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DEVELOPMENT AND CHARACTERIZATION OF GREEN SYNTHESIZED LAWSONE-ENCAPSULATED SILVER NANOPARTICLES FOR ANTICANCER APPLICATIONS

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ABSTRACT

Plants are used as medicines since time immemorial. India has a rich heritage of using medicinal plants in traditional medicines such as Ayurveda, Siddha and Unani besides folklore practices. In this study, an attempt was made to find out the anti bacterial potential of L. inermis leaves which are commonly used to treat various ailments. The leaves of Lawsonia inermis was extracted with three different solvents namely hexane, chloroform and ethyl alcohol. After that lawsone extracted and formulate silver nanoparticle via green synthesis process. The lowsone encapsulated silver nanoparticle was characterized via UV, FTIR, DSC, XRD, AFM, Drug release, and also then tested their activity against cancer cell.

Keywords: Lawsone, henna, silver nano-particles, cancer, drug delivery.

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INTRODUCTION

Several studies have shown that bio-active targeting nanoparticles significantly improve tumour cell selectivity and cytotoxicity when compared to traditional anticancer medicines and non-targeted nanoparticle techniques. Furthermore, it improves the drug's effectiveness and safety.

Cancer: Conventional and emerging therapeutics

Cancer is the second biggest cause of death in the world, after only heart disease-related mortality. In 2015, 8.7 million people died as a result of the 17.5 million cancer diagnoses registered worldwide. According to WHO fact sheets from 2018, lung cancer killed 1.69 million individuals, followed by liver cancer (788000 cases), colorectal cancer (774000 cases), stomach cancer (754000 cases), and breast cancer (754000 cases) (571000 cases). Cancer, in particular, kills one in every seven people worldwide, killing more people than malaria, TB, and AIDS combined. Surgery, radiation, and the use of chemotherapeutic medicines are all examples of traditional cancer treatment procedures that lack specificity. Surgical therapy cannot guarantee total cancer cell eradication, might be traumatic with big tumours, and is rendered useless in the event of sub-clinical metastases. Radiation treatment has dangers such as damage to healthy cells and tissues around the treated region, tiredness, and the development of secondary malignancies [1].

Chemotherapeutic medicines' many negative side effects, including the toxicity of their by-products, have a deleterious impact on non-targeted host cells. In addition, the development of innate and acquired resistance to these medications reduces the effectiveness of chemotherapy [2]. Inhibitors of angiogenesis, signal transduction pathways, and immunotherapy using mono clonal antibodies that interfere with biological processes governing tumour development and progression are examples of targeted treatments [3]. The US Food and Drug Administration approved 'gene modification therapy,' also known as adoptive cell transfer (ACT), in 2017, which involves taking T cells from a patient and engineering them to express a gene for a special receptor

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called a chimeric antigen receptor (CAR) that targets a specific protein on the cancer cell [4,5].

Nano technology and nano medicine for cancer cure

Despite the discovery and availability of a number of aggressive treatment options, cancer mortality rates continue to grow [6]. As a result, the development of effective, biocompatible, and cost-efficient cancer treatment approaches is critical. Because of their longer half-life and enhanced targeting efficiency, nanotechnology-based techniques have emerged as a fascinating area that promises to overcome the limits of traditional therapies [7]. Different factors, such as nanoparticle size, enable them to penetrate intrinsically into tumours through the increased permeability and retention (EPR) effect, their capacity to elude the immune system, and improve the medicine shalf-life; drastically lower their effective dosage. Because of their great surface density, nanoparticles may also be targeted selectively [8,9].

Nanoparticles (NPs) are atom clusters that vary in size from 1 to 100 nanometers. Nanomaterials are crucial because a metal's physico-chemical characteristics change when it shrinks to the nanoscale, allowing it to demonstrate features that are distinct from those of the bulk metal. The production of stable dispersions of nanoparticles, which are important in fields such as photography, catalysis, biological labelling, photonics, and optoelectronics, has mostly relied on gold, silver, and platinum [10]. Their synthesis was done using chemical, physical, and biological means. Chemical and physical techniques of NP fabrication are the most common, however they involve the use of toxic and dangerous chemicals, a lot of energy, and produce damaging waste [11].

Chemical and physical approaches both have negative environmental consequences in addition to being technically difficult and costly. As alternative, natural products such as sugars, biopolymers, microbes, and plant extracts containing phytochemicals are thought to play a crucial role as bio reductants in the conversion of metal ions to nanoparticles, as well as providing capping agents to stabilize them. Because maintaining aseptic conditions for microbial cultures is not industrially practicable, plant extract has been proven to be more favourable than microbial systems for large- scale synthesis of biogenic nanoparticles [12].

Biogenic gold, silver, copper, titanium, zinc, and iron generated from various bio-sources have been shown to have anticancer properties in several in vitro investigations on various cancer cell lines [13,14]. Due to its antibacterial, antifungal, larvicidal, anti-parasitic capabilities and industrial uses, silver nanoparticles (AgNPs) have grown in popularity and acceptance [15]. AgNPs are cytotoxic to cancer cells and have a lot of promise as an anti cancer drug because they cause cytotoxicity. The precise mechanism of AgNPs' anticancer action is yet unknown. However, the proposed mechanisms include the production of reactive oxygen species (ROS) that cause mitochondrial damage, induction of sub-G1 arrest in cells, up regulation of p53 protein, caspase-3 expression [16]. Inhibition of VEGF-induced activities particle size, surface area/reactivity, distribution pattern, and cell type specificity are all factors that influence cytotoxicity [17].

Lawsonia (family Lythraceae) is a monotypic genus represented by Lawsonia inermis linn (syn. L. alba Lam). It is a native of the North Africa and south west Asia widely cultivated as ornamental dye plant. Henna leaves are used as a prophylactic against skin disease. They have astringent properties and antioxidant properties. They are used externally in the form of paste or decoction against boils, burns, bruises, and skin inflammations. The leaves decoction is used as gargle for relaxed sore throat. The essential oil obtained from the flowers finds use in perfumery due to its β-ionone content. Henna was also reported to have tuberculostatic activity. The roots of this plant are useful in burning sensation, leprosy, stranguary and premature graying of hair. It is reported that Lawsonia inermis leaves possess antibacterial, antifungal, antiviral, anti- parasitic, anti dermatophytic, tuberculostatic, anti fertility, analgesic, anti- inflammatory activity, cytotoxic activity and enzyme inhibitory activities [18-30].

Silver nanoparticles are possible bioactive anticancer medications, with the ability to transport the drug to the appropriate cancer location. This strategy of mixing natural and manmade polymeric materials will open up new avenues for anti cancer medication delivery. The current study will provide an effective strategy for cancer illness management and therapy. Furthermore, this technology will aid in the development of innovative methods and bioactive molecule-NPs conjugation strategies for cancer therapy at low doses with fewer side effects. The advancements in the utilization of biocompatible and bio-related copolymers for cancer therapy have been productive, and will include their usage as delivery vehicles for effective anti-cancer medicines like laws one. In the near future, a new paradigm for the design of polymeric drug delivery systems will emerge by combining viewpoints from the synthetic and biological sectors [31-40].

Silver nanoparticles have been shown to enhance therapeutic efficacy, increase permeability, improve biodistribution, and reduce non-specific toxicity in anticancer medicines. AgNPs are attractive delivery vehicles because of their capacity to shield nucleic acids from degradation, their high biocompatibility, and their potential to deliver therapeutic compounds to cancer cells in vivo. Because of their unique intrinsic feature, nanoparticles provide excellent tumor- targeting carriers. Almost all cancers have inadequate lymphatic drainage and fenestrated vasculature, resulting in an increased permeability and retention (EPR) effect, allowing nanoparticles to concentrate at the tumour site in

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particular. Nanoparticles will also aid to prevent the reticulo endothelial system and mononuclear phagocytes from reabsorbing anticancer bioactive compounds. Overall, these findings show that nanoparticles have the ability to circulate for long periods of time. Nano-carriers are a crucial device in the treatment and cure of cancer and other illnesses because their design properties can be easily and effectively adjusted [41-45].

The goal of this study is to look into and create novel bioactive-based formulations for tumour targeting in cancer therapy. The newly developed formulation will be very beneficial in the prevention and treatment of cancer. The current study will provide an effective way for delivering bioactive chemicals to specific targets, which will aid in the effective treatment of cancer sickness while reducing side effects.

The use of silver nanoparticles (AgNPs) for the administration of an anticancer medication (lawsone) has been suggested, and this nano particulate system might provide various benefits, including:

The AgNPs system may deliver medication to tumour cells selectively through receptor mediated endocytosis. It has the potential to lower the dosage of anticancer medications. The delivery of anticancer drug "lawsone" for lung cancer targeting and therapy was suggested using silver nanoparticles of bioactive chemicals (produced by green synthesis).

Most of the herbal formulations and drug moieties, usnicacid, curcumin, piperine, and other strong anticancer bioactive chemicals have poor water soluble or lipophilic character, are less stable, have poor bio-distribution, and have low bioavailability. All of these medications have the potential to slow tumour development, but further study is needed to improve treatment effectiveness. In addition, natural bioactive materials must be delivered to the tumor's delivery location to boost bioavailability. According to several experts, nano-scale drug delivery systems have a high probability of effectively increasing and formulating the therapeutic efficiency of anticancer drugs. According to a review of the literature, silver nanostructure frameworks (AgNPs) constitute a distinct division of highly tailorable, well-designed materials. The nanoscale size promotes colloidal dispersion and increases surface area, allowing for finer control of the bioactive material's physicochemical characteristics.

Formulation and characterization of silver nanoparticles (AgNPs) for efficient tumour targeting, as well as increasing bioavailability, effectiveness, and reducing adverse effects of the herbal component "lawsone" derived from henna plant leaves (Lawsonia inermis). The formulation and characterization of AgNPs, as well as the improved formulation, will be compared to commercial goods, and the pharmacological effectiveness of the optimized formulation will be investigated.

MATERIALS & METHODS

Material

Fresh leaves of henna were collected from the premises of Department of Biological Science, Rani Durgawati University, Jabalpur, Madhya Pradesh, India. Silver nitrate and ethanol was obtained from Himedia Labotratory, Mumbai, MS, India.

Methods [45-60]

Collection of plant materials

Lawsonia inermis (Henna) Linn. leaves samples used in this study will be collected from Botanical Garden and then the specimen thus obtained will be identified and authenticated. The plant material (leaves of Lawsonia inermis) was air-dried at room temperature for 2 weeks, after which it was grinded to a uniform powder.

Solvents and Chemicals

Hexane, chloroform and ethyl alcohol will be obtained/purchased from any reputed chemical suppliers i.e. Himedia/Sigma/CDH and distilled and the purified form was used for extraction procedure in this study. Muller-Hinton broth and agar medium from Hi-media Pvt. Ltd. (Mumbai, India), will be used for testing antibacterial activity. Silica gel for column chromatography was Acme's silica gel (100-200 mesh). It will processed and activated by heating at 120°C for 1 h. Silica gel G containing 13% gypsum as binder will used for preparing TLC plates (20x5cm), layer thickness 0.5 mm. The plates will be activated by heating at 120°C for half an hour before use. Visualization will proceed either by exposure to iodine vapour and by spraying with 1:1 aqueous sulphuric acid and by heating at 110°C for 5 min.

Preparation of crude extract

The powdered plant material will be taken in three different aspirator bottles and successively extracted with solvents in the order hexane, chloroform and ethyl alcohol. After each extraction, the solvents were evaporated under reduced pressure using high vacuum conditions.

Bioactive compound isolation and characterization

Using chromatography and spectroscopic techniques, investigate the chemical ingredients found in plant extracts.

Preparation of 0.1M $AgNO_3$ Solution

To prepare 0.1 M $AgNO_3$ solution, first sufficient amount of water was taken in a cleaned and dried volumetric flask. About 1.69 g of silver nitrate will be added with continuous stirring, more distilled water was added, made up to the volume of 100 ml, and mixed thoroughly. The solution will then kept for at least one hour and then used for further processes.

Synthesis of silver nano particles

The prepared 0.1 M aqueous silver nitrate ($AgNO_3$) solution will be used for the synthesis of silver nano particles; 10ml of the extracted plant sample will be added into 90 ml of aqueous solution of 0.1M silver nitrate for reduction in to Ag+ ions and kept at room temperature for 5 hrs. After five hours of incubation, the change of yellow colour to brown colour indicated the synthesis of silver nanoparticles.

Characterization of Synthesized Silver Nanoparticles

Surface characteristics by AFM

The structure and surface characteristics of prepared nanoparticles was examined with AFM (SPM-9500, Shimadzu) in contact mode. The above mentioned AFM of the nanoparticles was done by Simicro cantilever with sample solution was spotted on mica and allowed to stand for a minute with substrate and blow off with air and observed for AFM photomicrograph by using SPM lab software.

Zeta potential and Particle size

The particle size and zeta potential of formulated nanoparticles is carried out by using Malvern instrument (DTS Ver. 4.10, Malvern Instruments, WR14 1XZ, UK). An appropriately diluted dispersion of AgNPs was placed in the compartment of a particle size an alyzer and finally average particle size and poly dispersity index were obtained. The zeta potential possessing the charge over the surface of particle indicates the colloidal system physical stability. The zeta potentials for the AgNPs were ascertained by Laser Doppler Anemometry (LDA) by employing the Zeta sizer (Malvern Instruments, UK).

Entrapment proficiency

Laws one encapsulated AgNPs (10 mg) was dissipated in solvent system (acetone). Primarily, the dispersion was centrifuged at 5000 rpm (cooling centrifuge) for about 15 min, remove the polymeric debris and then supernatant was collected. The clear supernatant solution was analyzed with HPLC at 260nm wavelength, (Wasters HPLC, Model-515) to calculate the amount of loaded lawsone in the prepared nanoparticle system.

In-vitro drug release study

Drug (lawsone) loaded AgNPs was filled in the dialysis bag (Hi media) separately and placed into separate 50ml of phosphate buffer saline (PBS) solution at a pH 7.4 with constant stirring at 100rpm in at 37±2°C. At a fixed time interval 1ml of buffer solution was withdrawn and replaced with the similar amount of fresh buffer solution. The amount of drug released from nanoparticles was analyzed by using HPLC system (Wasters HPLC, Model 515).

Hemolytic toxicity

For hemolytic activity whole human blood was amassed and collected in a collection vial. Firstly human blood was centrifuged at 10,000 rpm for 10 min for complete separation of RBC and plasma. The plasma was discarded and RBC was taken for further procedure. The RBC was re suspended in saline solution to form 10% hematocrit, then the red blood corpuscles (one mL) was incubated separately with 10mL of distilled water, (taken as100% hemolytic standard). In case of hematocrit solution with nanoparticle (drug solution),the laws one loaded AgNPs formulations was added separately on hematocrit solution (10% hematocrit) of distilled water upto 10 mL. The collection tube was allowed to stand for 1-2 hrs at 37°C, after that the drug loaded nanoparticle in hematocrit mixture was centrifuged at 5000 rpm for 5min, then the absorbance was taken of supernatant at 540 nm to optimize the effect of nanoparticle formulations against RBCs, which was useful to predict the percentage hemolysis.

SRB (sulforhod amine B) assay

SRB assay is an economic, swift, and sensitive process for calculating the cytotoxic potential of test substances, depended on content of cellular protein for adhered suspension culture in 96 well plates. This process is adequate normal laboratory purposes and for huge scale importance such as large output of anticancer drug screening (in vitro). The desired human lung cancer cell line (A549), was established in flask containing tissue culture medium and grown at adequate atmospheric condition temperature (37°C) and relative humidity (5% CO_2 and 90%) to get large amount of cells. After excavating cells from trypsin-EDTA treatment cell density was maintained to10,000 cells/100µl in suspension containing cells. The cell suspension (100µl) was poured to each well of 96 well plate by handy step process, and plates were incubated at 37°C, in an adequate atmosphere relative humidity (5% CO_2 and 90%) for 24 hrs, then after the sample (silver nanoparticle solution) was added to the wells of 96 well

plates at various concentrations (10µg/ml, 20µg/ml, 40µg/ml and 80 µg/ml).After 48 hrs of addition of sample, the plates were detached from incubator and trichloro acetic acid (TCA) was added in the concentration 50 µl of chilled 50%, in all the well of the plate to stop the reaction and made up the concentration10% and plates were incubated again at 4°C for 1 hr for fixation of the cell into the underneath of the wells. Plates were washed repeatedly with distilled water followed with air drying. The 100 µl dye solution of 0.4% in 1% acetic acid was poured to each well of the plate and left in room temperature for 30 mins at followed by washing with1% acetic acid and air dried after that 100µl (10.5M) of Trisbuffer was added in each well and shaked with mechanical shaker for 20mins. ELISA reader was used for recording optical density of cell at 540nm wavelength.

Statistical analysis

Final research outcome were showed as mean ± SD. This evaluation was achieved by t-test . $P<0.05$ was also important. All processes were performed thrice.

RESULTS & DISCUSSION

The green synthesis of silver nanoparticles using lawsone obtained from henna leaf extract was successfully carried out, as the change in the color of the solution from yellowish brown to dark brown color exhibits the reduction of the silver nitrate in aqueous solution due to excitation of surface Plasmon vibrations in silver nano

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particles. During this reaction process the pH of the solution changes from 5.93 to 5.72, which implies that the reaction occurs under acidic condition? This complete reaction occurs in 3-5 hours. The brown to dark brown color change of the reaction mixture indicated the formation of silver nanoparticles.

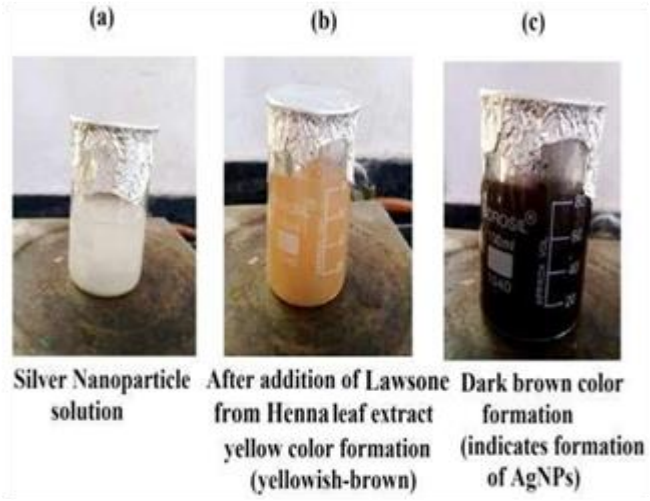

Figure 1: Synthesis of lawsoneen capsulated AgNPs of leaf extract of henna

Particle Size and Zeta Potential

Dynamic light scattering analysis suggested particles size of AgNPs showed them average size distribution in the range of 52.6nm (Figure 2) and zeta potential was found to be - 14.6mV (Figure 3).

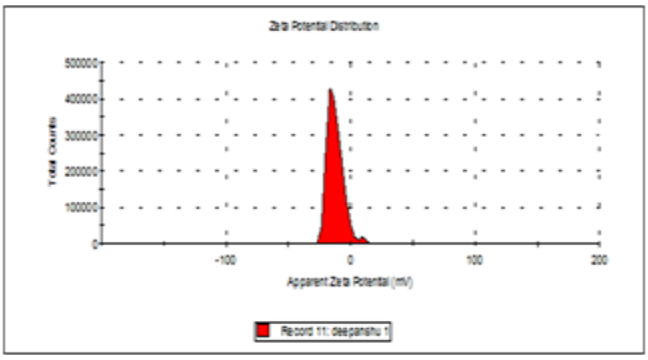

Figure 2: Particle size distribution of prepared AgNPs of lawsone.

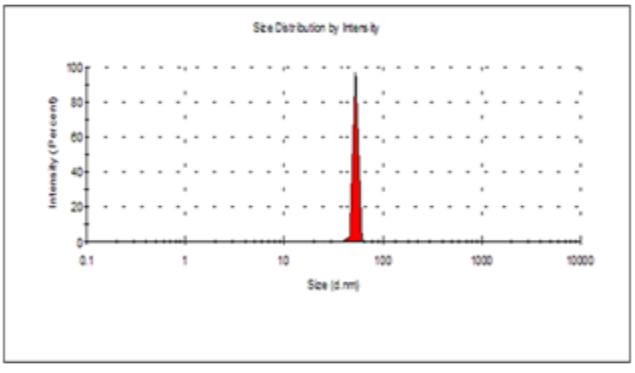

Figure 3: Zeta potential of prepared AgNPs of lawsone

Atomic Force Microscope Analysis

Atomic force microscopy (AFM) analysis is a commonly used technique for the determination of the size of NPs. AFM gives us in sight about the roughness of AgNPs. The size of metal NPs was observed from tip-corrected AFM measurements and the shape of AgNPs were determined. The tip-corrected measured the size of NPs in the range of 40-60 nm (Figure 4).

The surface morphology of prepared silver nanoparticles was depicted in Figure 4a and b. The Figure 4a, represent the 2D image of silver nanoparticle which represents the smaller size of particles whereas in Figure 4b, the 3D image of nanoparticle system was displayed, which suggested the uniform size distribution of the prepared system.

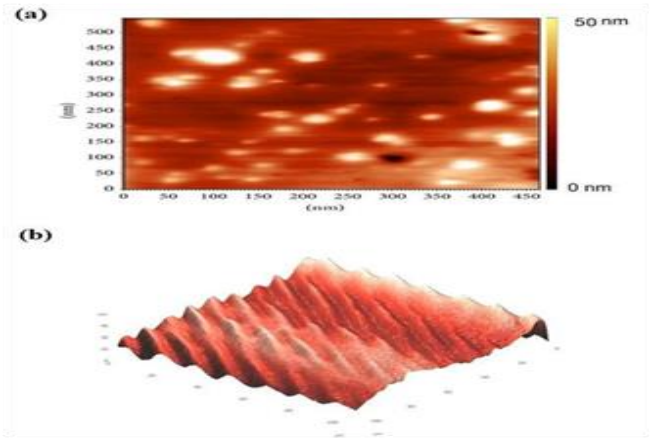

Figure 4: AFM of prepared AgNPs (a) 2D image; (b) 3D image

In-vitro drug release pattern

The graph depicts the drug's sustained and extended release from a nano particle system (Figure 5). The drug release graph depicts the regulated and long-term release of lawsone from the silver nanoparticulate systems, respectively. Plain lawsone were liberated 94.66% in 12 hours, while lawsoen from Ag-nano particles were maintained for 48 hours and released 97.89 %.

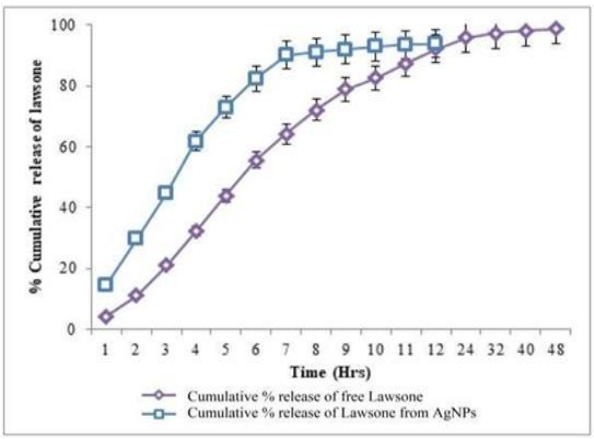

Figure 5: Percentage cumulative release of free lawsone and lawsone from AgNPs

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Hemolytic toxicity study

A hemolytic toxicity research was conducted to determine the hemotoxic impact of the designed Ag-nanoparticles. Plain lawsone and lawsone loaded AgNPs, showed hemolytic toxicity of up to 30.25±1.50%, and 7.89±0.95%, respectively (in distilled water) (Table 1). Plain lawsone and lawsone loaded Ag-nanoparticle formulations containing 0.1µM equivalents of lawsone. The content of medicines was used to assess the lawsone Ag-nanoparticle formulation. Hemolytic toxicity was reduced as a result of the nanoparticls' delayed release of encapsulated drug molecules. According to the results of a hemolytic toxicity investigation, lawsone loaded AgNPs showed less hemotoxicity than plain lawsone.

Table 1: Absorbance of hemolytic standard at 540 nm.

S. No.	Hemolytic Standard	Absorbance at 540 nm
1	Hemolytic Standard of distilled water	0.1995

Table 2: Data of absorbance of 10% hematocrit solution at 540 nm.

S. No.	10% hematocrit solution	Absorbance at 540nm	Toxicity
1	10% hematocrit solution of distilled water with lawsone	0.1918	30.25±1.50
2	10% hematocrit solution of distilled water with lawsone loaded AgNPs	0.1810	7.89±0.95

SRB (sulforhod amine B) assay

The SRB test was used to establish in-vitro cytotoxicity screening of lawsone encapsulated silver nanoparticles in the A549 human lung cancer cell line. The assay's results confirm a dose- dependent evaluation of cytotoxicity, with cellular bioavailability decreasing as sample concentration increased. AgNPs that have been loaded with lawsone. Figure (Fig. 6) shows the consequence of the cell's percentage growth inhibition. Higher concentrations of lawsone limit cell development, according to the findings. Furthermore, when the concentration of lawsone increases, cell viability decreases. Lawsone is available in free form or encapsulated form in silver nanoparticles. When compared to ordinary lawsone, and AgNPs formulations were shown to be cytotoxic to a larger extent at concentrations between 10-80 µg/ml.

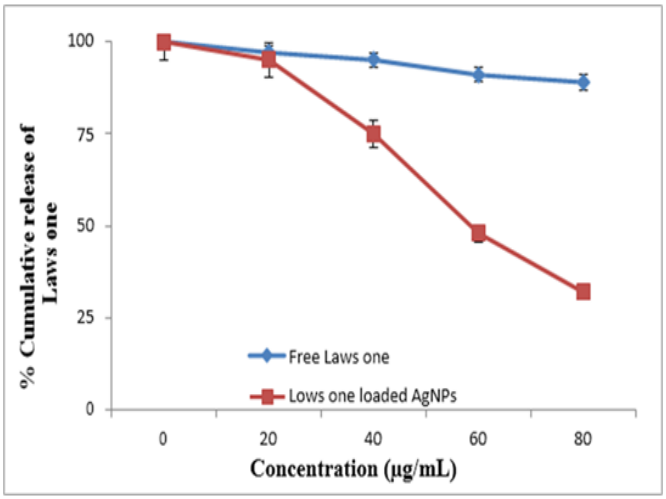

Figure 6: In-vitro percentage control growth of lawsone loaded AgNPs in A549 Cancer cell line

The percentage growth inhibition of the cell (A549) demonstrated that greater lawsone concentrations impede cell growth. When compared to free lawsone, NPs formulations were shown to be cytotoxic to a larger extent at concentrations between 10-80 µg/ml.

CONCLUSION

Silver nanoparticles are possible bioactive anticancer medications, with the ability to transport the drug to the appropriate cancer location. This strategy of mixing natural and manmade polymeric materials will open up new avenues for anticancer medication delivery. The current study will provide an effective strategy for cancer illness management and therapy. Furthermore, this technology will aid in the development of innovative methods and bioactive molecule-NPs conjugation strategies for cancer therapy at low doses with fewer side effects. The advancements in the utilization of biocompatible and bio-related copolymers for cancer therapy have been productive, and will include their usage as delivery vehicles for effective anti-cancer medicines like lawsone. In the near future, a new paradigm for the design of polymeric drug delivery systems will emerge by combining viewpoints from the synthetic and biological sectors.

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