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Gum Acacia (GA) Coated Algal Mediated Biogenic Silver Nanoparticles Synthesis (GA-AgNps) for the Potential Pharmacological Activities

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In the present study, silver nanoparticles synthesized from cold tolerant strain of *Spirulina platensi* was functionalized with gum acacia (GA) and the functionalized silver nanoparticles (GA-AgNps) were evaluated for pharmacological activities. Functionalization was confirmed by UV visible spectrophotometry,scanning electron microscopy equipped with energy dispersive atomic X ray spectroscopy,fourier transform infrared spectroscopy and x ray diffraction studies.Pharmacological activities such as anti bacterial activity was studied against human pathogenic bacterial strains *Pseudomonas aeruginosa* and *Staphylococcus aureus* adopting well diffusion assay,microtitre plate spectrophotometric mediated biofilm inhibition assay and anti cancer activity against human liver carcinoma (Hep 2) cell line by MTT assay.All the characterization revealed the functionalization of nanoparticles and the functionalized GA-AgNps showed distinct anti bacterial activity at all the concentration against tested bacterial strains. *In vitro* anti cancer activity of functionalized nanoparticles was evaluated against human Hep 2 cell lines adopting MTT assay which reveals that cell viability has been reduced as dose dependent manner.The present study would suggests the possible utilization of gum acacia stabilized silver nanoparticles (GA-AgNps) as an effective chemotherapeutic biocide against human pathogenic bacteria and cancer.

Keywords: *Spirulina platensis*, Gum acacia, Silver nanoparticles, Functionalization,anti bacterial,anti cancer.

Synthesis of noble metal nanoparticles for various applications in science and technology is an area of constant interest. Gold, silver, and copper have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in various discipline of science[1,2]. Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increased attention due to a growing need to develop environmentally benign technologies in material synthesis[3] Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly,easily scaled up for large scale synthesis, where there is no need to use high pressure, energy, temperature and toxic chemicals[4].

Silver nanomaterials are antimicrobial agents and more effective due to their large surface area and high reactivity compared to a bulk solid, nanosized metal particles which exhibit excellent physical, chemical and biological properties[5]. Silver nanoparticles are synthesized by different physical and chemical methods like sol-gel technique, solvo-thermal synthesis, chemical reduction, laser ablation, inert gas con-densation etc. Biological synthesis of silver nanoparticles using plants and microorganisms are now extensively studied because of best biocompatibility and bioefficacy[6,7,8].

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Functionalization of nanoparticles is necessary for their stability, functionality, and biocompatibility. Functionalization is essential to preserve the properties of the silver and the bound biological molecule[9]. In other words, the biological molecule should be stable and able to retain its bio recognition properties and nanoparticles should be able to retain their unique properties such as strong plasmon absorption bands[10] light scattering[11] etc. For biomedical applications, surface-functionalization of AgNPs is essential in order to target them to specific disease areas and allow them to selectively interact with cells or biological molecules. In general, functionalization of can be performed by either using chemical functional groups or biological molecules. In the present study, gum acacia functionalized biogenic silver nanoparticles synthesized from cold tolerant strain of *Spirulina platensis*

There are some natural polymers such as chitosan and gum acacia have been reported as a polymer-based protective agent to stabilize the metal nanoparticles. Gum Acacia is a natural polymer derived from acacia trees. Due to its well steric stabilization effect as adsorbing on the surfaces of colloids and its numerous functional groups such as carboxylate and amine groups, it has been widely used as emulsifiers and capping agents[12,13,14,15]. Because of the biocompatibility, biodegradability, nontoxicity and adsorption properties, they were used as a stabilizing agent to prepare various metallic nanoparticles[16,17,18]. These polymer protected nanoparticles can be easily integrated into systems relevant for pharmaceutical, biomedical, and biosensor applications[19,20]. The present study is undertaken to evaluate gum acacia functionalized biogenic silver nanoparticles synthesized from cold tolerant strain of *Spirulina platensis* and their potential pharmacological activities such as anti bacterial, anti cancer activity.

MATERIALS AND METHODS

Reagents and media

Gum acacia (analytical grade) was obtained from Sigma– Aldrich Chemical Co.Tryptic soy broth, Muller Hinton agar,trypticase soy broth and agar, RPMI media, Fetal bovine serum (FBS), Trypsin, methylthiazolyldiphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from Hi media, Mumbai, India.

Synthesis of gum acacia functionalized Silver nanoparticles

Silver nanoparticles were synthesized by our previous work from cold tolerant strain of *Spirulina platensis*[21]. Algal mediated silver nanoparticles thus obtained were used for functionalization study.10 ml of purified silver nanoparticles in 50 ml of de ionized water was mixed with 5 ml of gum acacia solution (1%)and kept under magnetic stirrer at 35ºC for 3 hours to obtain homogeneous solution. The slurry thus obtained was centrifuged at 10000 rpm for 10 minutes. Collected pellet was lyophilized and used for further studies.

Characterization

Functionalization of silver nanoparticles with gum acacia was characterized by UV–vis spectrophotometery for the determination of surface Plasmon absorption maxima using Shimadzu-1800 spectrophotometer. Fourier Transform Infrared Spectoscopy (FTIR) was carried out with KBr palletized dried sample in the range of 4000–500 cm^{-1} using Bruker OpticGmbH Tensor 27.Crystalinity status was confirmed by X-ray diffraction (XRD) measurement using Rigaku smart lab instrument operated at a voltage of 40 kV and a current of 30 mA with Cu Ka1 radiations. Particle morphology (Shape and size) and elemental composition was studied by field emission scanning electron microscopy equipped with energy dispersive X-ray analysis (FESEM–EDAX) was performed by SUPRA 55-CARL ZEISS,Germany.

Evaluation of pharmacological activities

Anti bacterial activity

Bacterial strains

Anti bacterial activity was studied against human pathogenic bacteria *Pseudomonas aeuroginosa* (ATCC 10145) and *Staphylococcus aureus* ATCC 12123).Both the strains were obtained from American Type Culture Collection (ATCC). Pure culture of the both strains were maintained on Tryptic soy agar (TSA) slants.

Inocula preparation

Both the bacterial **c**ultures were inoculated from tryptic soy agar (TSA) slant into 25 ml of tryptic soy broth,incubated under shaking condition at 37 ° C for 24 hours.

Well diffusion assay

Anti bacterial activity of the tested bacterial strains was studied by well diffusion assay. Inocula of the respective bacterial culture thus prepared was uniformly spread with sterile cotton swabs on sterile Mueller Hinton (MH)Agar Media (Hi-media, India). The wells were made using cork borer and aliquots of silver nanoparticles (aliquots of 25, 50, 75 and 100 µg/ml were prepared from concentrated nanoparticles) was loaded into the wells. The plates were incubated at 37ºC for 24 hours.After the incubation period,the plates were observed for zone of inhibition.Three replicates were maintained.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibition concentration (MIC) was determined by a turbidimetric method[22]. Bacterial inocula prepared in tryptic soy broth as earlier was used in this study. In this method, a series of 5 ml of screwcap vial (Borosil) each containing 2mL broth medium was prepared. 2mL of Nanoparticles suspension with different concentration were prepared and added into the first vial.After mixing, 2 mL of mixture from this vial were transferred to the next vial, and a similar procedure was repeated for the subsequent vials. The bacteria inocula was added to the vial to achieve a final bacterial concentration of 10^5 cells/ mL Inoculated vials were incubated under shaking condition (150 rpm/min) at 37°C for 20 hours. The MIC was determined as the minimum concentration at which there is no visible change in the turbidity of the medium. The minimum bactericidal concentration (MBC), defined as the lowest concentration of sample that kills 99.9% or more of the initial inoculum, was determined in those test samples after the MIC test showed no growth. The assay was carried out by counting the number of colonies after the bacteria were seeded overnight on agar plates. The MIC and MBC were determined in a similar fashion. Triplicates were maintained in each treatment.

Evaluation of biofilm inhibitory effect

Biofilm inhibitory effect of gum acacia stabilized silver nanoparticles was studied against tested bacterial strains by crystal violet spectrophotometric microtitre plate assay[23,24]. Respective bacterial inoculum was prepared in tryptic soy broth as described earlier and 100 µL of respective bacterial inoculums was transferred to the 96 well microtitre plate under aseptic condition. 100 µL of nano suspension with different concentration was added to the bacterial inoculm and the microtitre plate was incubated at 37°C for 48 hours. After the incubation period, the content in the well was completely removed and the wells were washed with phosphate buffered saline (PBS) followed by sterile distilled water.After washing, 100 µl of 0.1% aqueous solution of crystal violet was added,incubated at room temperature for 30 minutes .Followed by the incubation period, crystal violet was removed and washed using sterile

Table 1. Zone of inhibition (mm) of GA-AgNps against tested bacterial strains

S. No.	Concentration (µg/ml)	Zone of inhibition (mm) *Pseudomonas aeruginosa*	*Staphylococcus aureus*
1	25	13.0	13.2
2	50	13.0	13.4
3	75	14.0	14.0
4	100	14.0	14.0

Mean value of three replication is not statistically significant (P>0.05 level) by DMRT

Table 2. MIC and MBC of GA –AgNps against tested bacterial strains

S. No	Tested bacterial strains	MIC (mg/ml)	MBC (mg/ml)
1	*P.aeruginosa*	0.34	0.52
2	*Staph.aureus*	0.41	0.71

Mean value of three replicates

Table 3. Biofilm inhibition (%) of GA-AgNps against tested bacterial strains

S. No	Concentration (µg/ml)	Biofilm inhibition (%) *Pseudomonas aeruginosa*	*Staphylococcus aureus*
1	25	30.0	33.3
2	50	51.0	54.0
3	75	78.0[a]	79.0[a]
4	100	97.4[a]	98.2[a]

Column carrying the same letter is statistically significant (P>0.05 level) by DMRT

distilled water.200 µL of 95% ethanol.was added to the wells,incubated for 15 minutes at room temperature.Absorbance of the ethanol solubilised mixture in the well was read at 540 nm in an UV-Visible spectrophotometer. Control and triplicates were maintained.

Anti cancer activity

Anti cancer activity of synthesized nanoparticles was studied against Hep2 cell line adopting MTT assay. Cytotoxicity of silver nanoparticles was carried out by inhibition of cell growth of Hep2 cell line using a tetrazolium dye (MTT) assay and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the control.

Cell line and growth condition

Human carcinoma cell line - Hep2 was derived from National centre for cell sciences(NCCS),Pune,India.The culture was routinely maintained at 27°C using RPMI1640 media under humidified atmosphere (5% CO_2).

MTT assay

Cells were collected six days after sub culturing and diluted with fresh medium to a density of $5x10^3$ cells/ml and transferred to the 96-well microtiter culture plate. followed by the addition of 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8 µg/ml concentrations of nanoparticles.Triplicates and control was maintained in each concentration. Seeded microtitre plate was incubated for 24hrs at 5 % CO_2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl—tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the nanoparticles on the proliferation of Hep 2 cells was expressed as the % cell viability,

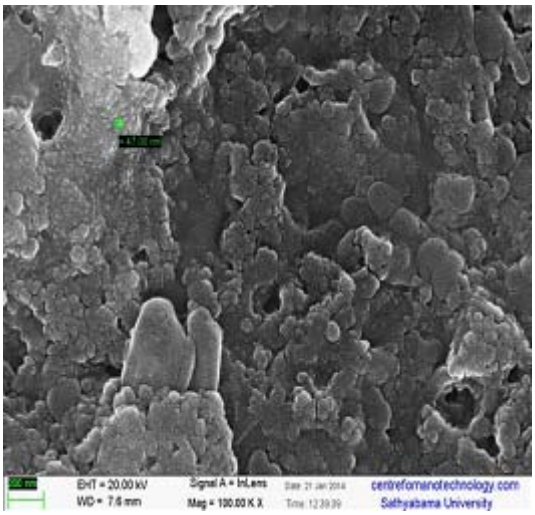

Fig. 1. Scanning electron microscopic image of GA stabilized AgNps

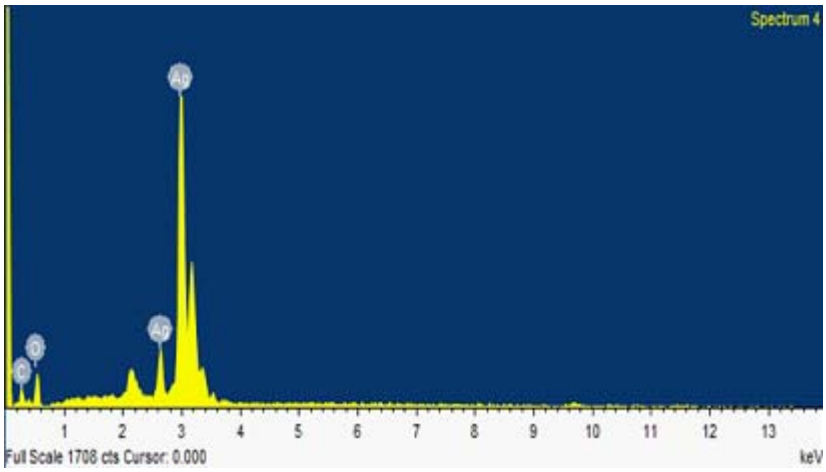

Fig. 2. Energy dispersive atomic X ray (EDAX) spectroscopic image of GA-AgNps

RESULTS AND DISCUSSION

The application of nanoparticles as delivery vehicles for bactericidal and anti cancer agents represents a new paradigm in the design of antibacterial therapeutics. Functionalization of the nanoparticles can be carried out by the polymers which in turn improves the stability and biocompatibility[6]. Primary confirmation of GA stabilized silver nanoparticles using UV-visble absorption spectrophotometry analysis reveals a broad surface plasmon absorption maxima at 430nm and remain in the same range at increasing incubation period (72 hours) suggesting nanodispersive particles in the aqueous solution. Particles morphology using scanning electron microscopy (SEM) with EDAX revealed spherical particles with the electron dense core shell with the size of 47 nm and all the elemental composition (Figure 1,2).Further characterization was carried out using FTIR.The FTIR spectra of Gum acacia exhibited characteristics bands at (Figure 3). X-ray –diffraction (XRD) measurement was used to elucidate crystallinity and the lattice properties of the synthesized nanoparticles. Presence of distinct high diffraction peaks indexing the Bragg's reflection planes confirmed the face centered cubic structure of crystalline nanoparticles. (Figure 4)

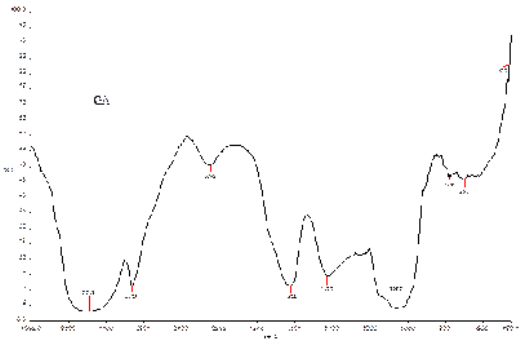

Fig. 3. FTIR analysis of GA

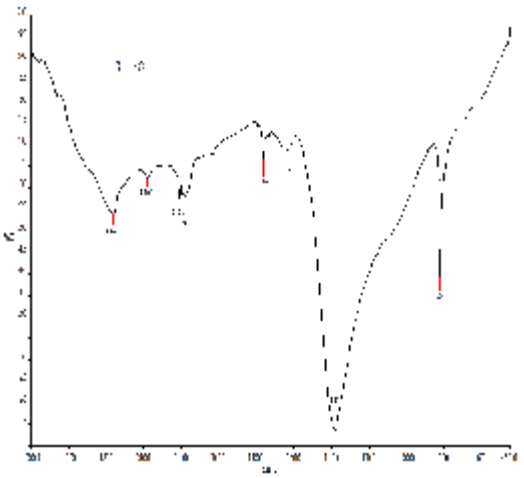

Fig. 4. FTIR spectra og GA- AgNps

Pharmacological activity

In the present study,potential pharmacological activities such as anti bacterial and anti cancer activity of the synthesized GA stabilized silver nanoparticles has been studied against human pathogenic bacterial strains and Hep 2 cell line respectively.Anti bacterial activity adopting well diffusion assay revealed all the

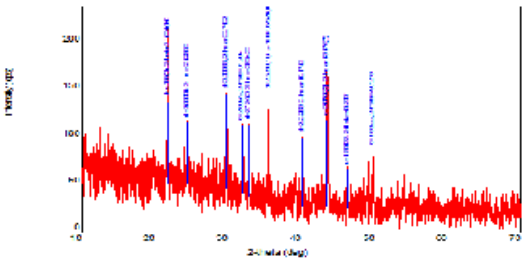

Fig. 5. XRD spectra of GA-AgNps

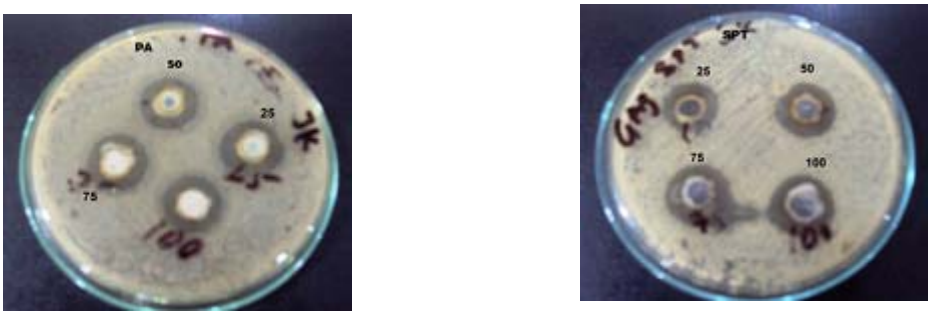

Fig. 6. Zone of inhibition of GA-AgNps against *P.aeruginosa* (a) *Staph.aureus* (b)

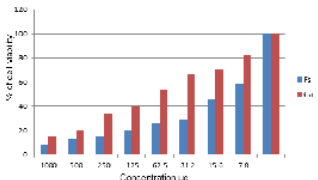

Fig. 7. Comparison of silver nano particles and gum acacia stabilized silver nanoparticles on % viability of Hep 2 cell lines

tested concentration inhibited both the tested strains (Figure 5). In the case of *P.aeruginosa*, the zone of inhibition was found to be 13.0,13.0,14.0 and 14.0mm.Similar finding was reported in *Staph.aureus*.13.2,13.4,14.0 and 14.0mm of zone of inhibition was recorded in the respective concentration (Table 1). MIC and MBC of the GA-AgNps against both the tested bacterial strains

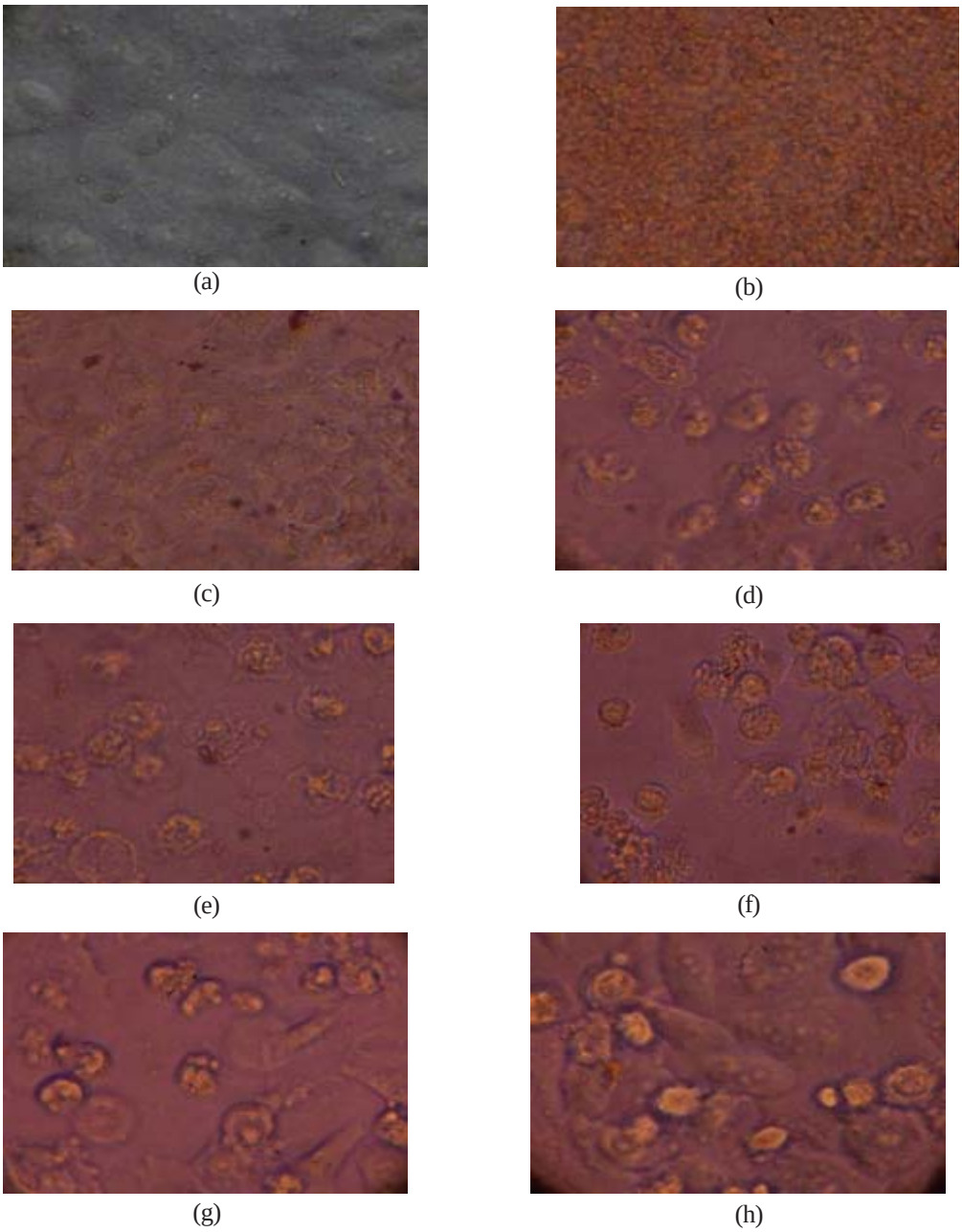

Fig. 8. Microscopic examination of gum acacia coated Ag Np's treated with Hep2 Cell Lines. a)1000 b) 750 c) 500 d) 250 e) 125 µg/ml f) control

was presented in Table 2 Anti bacterial activity was also studied with biofilm inhibition. In the present study,inhibitory effect of GA stabilized silver nanoparticles against biofilm of *P.aeruginosa* and *Staph.aureus* was studied by microtitre plate spectrophotometric assay. This study revealed the biofilm of both the tested strains was inhibited by the nanoparticles treatment as dose dependent manner. In general, maximum inhibition was recorded in 100 µg/ml concentration against the both tested strains.30.0,51.0,78.0,97.4 % and 33.3,54.0,79.0,98.2% of inhibition was recorded in *P.aeruginosa* and *Staph.aureus* at the respective concentration (Table 3).

The *in vitro* cytotoxicity effects of gum acacia stabilized silver nanoparticles synthesized from *spirulina platensis* were screened against human liver carcinoma Hep 2 cell lines adopting MTT assay to determine the anti cancer activity.A serial 10 fold dilution of gum acacia stabilized silver nanoparticles was incubated with Hep 2 cells grown in 96 well plates and the viability in respective dilution was determined by MTT assay which reveals that all concentration if diluted nanoparticles reduced the viability Effective high cytotoxic effect was recorded at 1000 µg/ml which showed 19.6% of viability followed by 750 and 500 µg/ml (23.0 and 27.3 %). A gradual increase in viability was observed in further decreased concentration (Figure 6).Further cytotoxicity was determined by studying morphological changes of the nanoparticles treated cells under inverted microscope (Figure 7).It can be seen that high concentration of nanoparticles (1000 and 750 µg/ml) caused distinct cell disruption No changes in morphology was observed in less dose of nanoparticles treatment.

The present study clearly reveals effective anti bacterial and anti cancer agent formulated from the algal mediated biogenic silver nanoparticles functionalized with gum acacia would suggests the possible utilization of GA-AgNps formulation as an effective therapeutic agents

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