Bioconjugation of Iron Nanoparticles with the Enzyme L-asparaginase Synthesised from *Escherichia coli* for Cancer Drug Delivery

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Abstract:
L-asparaginase is an anti-neoplastic agent used in the lymphoblastic leukaemia chemotherapy. The present work deals with L-asparaginase activity from *E. coli* was detected on a M-9 medium plate showed colonies which have exhibited clear pink zone and Czapek Dox’s Medium agar tubes was changed from yellow to pink after 48 hrs. Production of L-asparaginase by submerged fermentation was carried out using modified M9 broth medium. The enzyme was purified to near homogeneity by ammonium sulphate precipitation, dialysis, Column chromatography using Sephadex G-100 and SDS-PAGE. The enzyme was purified 5.24 U/mL. SDS PAGE of the purified enzyme revealed with apparent molecular weight of 110 kDa. Synthesize of iron nanoparticles by using chemical methods and bioconjugation of L-asparaginase with Iron Nano Particles followed by APTES modification of magnetic iron oxide nanoparticles, glutaraldehyde activation and immobilization of L-asparaginase on the MNPs. The obtained L-asparaginase coated iron nanoparticle and iron nanoparticles were characterized by UV-vis spectroscopy, FTIR and TEM. TEM image of the iron nanoparticles shows particle size range 6.58 nm and enzyme coated MNP was at a range of 500 nm. Finally anti-cancer properties of a therapeutic drug studied L-asparaginase, iron nanoparticles and L-asparaginase enzyme coated iron nanoparticles through normal cell lines and HeLa cells lines using MTT assay revealed higher cell death in HeLa cell lines than normal cell lines. In HeLa cells lines 91% of viability of the test drug even at the minimum concentration of 1μl further when compared to the anti-cancer drug L-asparaginase and iron nanoparticles. The Metallo-proteins or the Metal-asparaginase nanobiocomposites exhibited higher Anti-Cancerous activity than the enzyme L-asparaginase or iron nanoparticles alone.

Keywords: L-asparaginase, *Escherichia coli*, Iron nanoparticles, L-asparaginase enzyme coated iron nanoparticles, MTT assay

1. INTRODUCTION

Microbial therapeutic enzymes play a major role in the biochemical investigation, diagnosis, curing and monitoring of many dreaded diseases. They are also used for diagnostic, scientific and analytical purposes. Typical examples of oncolytic enzymes are L-asparaginase, L-glutaminase, etc. The therapeutic enzyme L-asparaginase has attracted much attention as an amino acid degrading enzyme exhibiting antineoplastic activity [19]. The discovery of asparaginase a medicinal agent for the treatment of malignant tumors, was made in 1922. It is well demonstrated that only L-asparaginase obtained from *Escherichia coli* and *Erwinia chrysanthemi* have been used in humans. L-asparaginase, the enzyme which converts L-asparagine to L-aspartic acid and ammonia has been used as a chemotherapeutic agent. *E. coli* L-asparaginase under the brand name Elspar has been used for the treatment of acute lymphocytic leukemia for more than 20 years [21]. It can be given as an intramuscular, subcutaneous, or intravenous injection without fear of tissue irritation. Nanoparticles and nanotechnology have been increasingly used in the field of cancer research, especially for the development of novel approaches for cancer detection and treatment Magnetic iron oxide (IO, i.e. Fe₃O₄, γ-Fe₂O₃) nanoparticles (NPs) are particularly attractive for the development of biomarker-targeted magnetic resonance imaging (MRI) contrast agents, drug delivery and novel therapeutic approaches, such as magnetic nanoparticle-enhanced hyperthermia. Chemical synthesis of magnetic iron oxide nanoparticles by using co precipitation method. This method can be ferrous hydroxide suspensions are partially oxidized with different oxidizing agents. It consists in ageing stoichiometric mixtures of ferrous and ferric hydroxides in aqueous media, yielding spherical magnetite particles homogeneous in size. Being highly susceptible to oxidation, magnetite (Fe₃O₄) is transformed to maghemite (γFe₂O₃) in the presence of oxygen. Cancer is defined as uncontrolled division of cells. ALL is cancer of WBC, characterized by the excessive multiplication of malignant and immature WBC (lymphoblast) in bone marrow. In this regard, synthesis of bioconjugate nanoparticles using biological macromolecules [12] [13] has recently aroused great interest due to the broad range of applications of such hybrid materials, from life sciences to materials and nanosciences. Enzyme - nanoparticle conjugates that take advantage of the catalytic ability of bound enzymes have been reported for bioanalytical and biotechnological applications [22]. One extremely useful route to post-synthetic modification of iron oxide nanoparticles is accessed by employing the common organosilane reagent, γ-aminopropyl triethoxy silane (APTES) [23]. This approach has been used to produce polymer grafted magnetic nanoparticles (MNPs) [7] [8]
and to attach proteins to iron oxide nanoparticles. Nanoparticles containing drugs are coated with targeting agents. The nanoparticles circulate through the blood vessels and reach the target cells. Drugs are released directly into the targeted cells. Approximately 12.5 million new cases of cancer are being diagnosed worldwide each year and considerable research is in progress for drug discovery for cancer. Role of drug delivery in the management of cancers of the brain, the bladder, the breast, the ovaries and the prostate are used as examples to illustrate different approaches both experimental and clinical. The market value of drug delivery technologies and the anticancer drugs are difficult to separate.

2. MATERIALS AND METHODS

2.1 Microbial Synthesis of L-Asparaginase
In this present work the synthesis of L-asparaginase were performed by using bacteria [16].

2.1.1 Biological Strain and Growth Conditions for E. coli Culture
The bacterial strain used throughout the study for the production of L-asparaginase was E. coli. It was procured from Vivek Laboratories, in Nagercoil (India). The microbial strain E. coli was streaked on the surface of sterilized Eosin Methylene Blue agar plates and incubated at 37°C for 24 hours. The colonies possessing green metallic sheen were isolated, purified and preserved on Nutrient agar slants.

2.1.2 Determination of L- Asparaginase Activity
2.1.2.1 Qualitative plate assay for screening of L-asparaginase production
E. coli culture were inoculated into modified M-9 medium and screened for the potential producers of L-asparaginase using modified M-9 medium plate method. The single discrete colonies which have exhibited clear pink zone surrounding microbial colonies after 48 hrs incubation indicate L-asparaginase producing cultures. This can easily be detected by the change in pH of the medium using phenol red. These colonies (L-asparaginase positive cultures) were picked up and grown in the modified Czapek Dox agar [15].

2.1.2.2 Qualitative tube assay for screening of L-asparaginase production
Sterilized Modified Czapek Dox’s Medium (pH 6.8) 10 ml was distributed in the presterilized culture tubes to prepare stabs. After that a loopful E.coli culture was inoculated on the surface of solidified stabs and incubated at 37°C for 48 hours. Uninoculated stab was regarded as a negative control. Stabs were examined for change in colour of medium from yellowish to pink due to change of pH indicating the positive L-asparaginase activity.

2.1.2.3 Production of L-asparaginase by submerged fermentation
L-asparaginase production was done on modified M9 broth medium (pH 7) supplemented with L-asparagine (1% w/v), 35 ml of medium was taken in a conical flask of 150 ml and inoculated with 1 ml of E.coli culture and incubated at 37°C for 48 hours. After completion of the incubation period, culture broth was centrifuged at 5,000 rpm for 20 min at 4°C.

Agar well diffusion technique
The 100 µl of culture was poured into the agar well of diameter 8 mm prepared in plates containing modified Czapek Dox’s Medium. The filtrate was allowed to diffuse into the medium for 48 hrs at 37 °C. The diameter of zone (mm) of L-asparaginase activity, as indicated by the formation of pink colored zone around the well against the yellow background, was measured.

2.1.2.4 Quantitative assay of L-asparaginase activity
L-asparaginase activity was measured by a standard method using Nesseler’s reagent [10]. The reaction mixture containing 0.2 ml of 0.05M Tris-HCl buffer (pH 8.6), 1.7 ml of 0.01M L-asparagine and varying amount of enzyme solutions (100µl) to a final volume of 2 ml were incubated for 10 minutes at 37°C. After incubation, the reaction was stopped by adding 0.5 ml of 1.5M TCA. The contents were centrifuged at 10,000 rpm for 10 minutes and the pellet was discarded. To the supernatant, 1 ml of Nesseler’s reagent and 7ml of water was added, the absorbance was read at 480 nm using distilled water as blank. The enzyme activity was expressed in International unit.

International Unit (IU)
One IU of L-asparaginase is the amount of enzyme which liberates 1 mol of ammonia per minute per ml [mole/ml/min].

2.1.3 Purification Of L-Asparaginase:
After centrifugation of production media supernatant was collected and the volume of it was measured and subjected to salting out with ammonium sulphate (enzyme grade, SRL) at a final saturation of 80% ( w/v). The precipitate was recovered by centrifugation at 10,000 rpm for 15 minutes at 4°C. The pellet was dialyzed against phosphate buffer 9 (pH 7.4. 0.02M) using dialysis membrane (Dialysis membrane 60, Himedia) and subjected to column chromatography.

Column chromatography using Sephadex G-100
Sephadex cellulose (Sigma aldrich) was used as the column material which was suspended in a large volume of phosphate buffer (pH 7.4). The gel was swollen by incubating overnight in phosphate buffer. Good slurry of the gel was filled in the column without the trapping of any bubble. The gel was settled down and washed with phosphate buffer. The column was allowed to equilibrate thoroughly by passing through the column buffer. Applied the dialyzed sample on the top of the column bed. The sample volume was preferably limited to 1-3% of the total bed volume. The sample was applied by careful pipetting and was kept at -4°C for 2-3 hours for incubation. After incubation the unbounded proteins were eluted out with 10ml of 0.02M phosphate buffer. The bound proteins were eluted with gradations of 10 ml of phosphate buffer and nearly 8 fractions were collected. The fractions were collected and tested for L-asparaginase activity. The active fractions were stored at -4°C. The whole process was carried out at -4°C. A graph was plotted with concentration of enzyme units along x-axis and optical density along y-axis. From the graph, which fraction have maximum L-asparaginase activity.

2.1.3.1 Characterization of purified L- asparaginase
Determination of molecular mass and purity
Molecular mass of purified L - asparaginase was determined by SDS-PAGE. SDS-PAGE is the most widely used method for quantitatively analyzing any protein mixture, monitoring protein purity and to determine the molecular weight. It is based on the separation of protein according to their size and then locating them by binding to a dye. The glass plates were set with appropriate spacers and were sealed to avoid leakage. They were kept in vertical position by using clips. The glass plates were casted with 12.5% resolving gel and 5% stacking gel. After 10 to 15 minutes the comb was removed and the wells were washed with water. The assembly was transferred to electrophoretic
apparatus. 20µl of the sample was mixed with 20µl of loading buffer and boiled for 8 minutes in a boiling water bath. 20µl of the sample was loaded into corresponding wells equally, and the rest of the wells were kept free. The apparatus was connected with power supply and turned on. After the dye front had reached the bottom, power supply was turned off and the gel, was removed and stained in coomassive brilliant blue for overnight and kept in destaining solution for 2 hours. % viability was calculated for purified protease enzyme.

Rf = Distance travelled by protein / Distance travelled by the tracking dye

2.2 Synthesis of Iron Nanoparticles by Chemical Method

Iron nanoparticles were chemically synthesized using 8 ml of 0.1 M of FeCl$_3$ and 2 ml of 0.2 M FeCl$_2$ to a 100ml beaker and stir with magnetic stirring bar. 50 ml NaOH was added drop by drop, a brown colored precipitate was formed initially with ferrous and ferric hydroxide dehydrates giving brownish precipitates. These iron nanoparticles showed attractive property when a strong magnet was moved along the test tube indicating the presence of iron nanoparticles [18].

2.3 Bioconjugation of L-Asparaginase with Iron Nano Particles

2.3.1 Protocol for APTES modification of magnetic iron oxide nanoparticles

1.5 g Iron nanoparticles in 45 mL of double distilled water in a beaker and suspend the γ-Fe2O3 particles in aqueous solution was sonicated for 20 minutes. 5 ml APTES was added drop by drop to the solution under magnetic stirring for 3 hours. The reacted suspension was decanted and MNPs were separated using round magnet and separated MNPs were washed using double distilled water and ethanol. The resulting magnetic nanoparticles were dried over night in vacuum and stored for further usage [6].

2.3.2 Protocol for glutaraldehyde activation

1 ml of PBS was added to 10 mg APTES –modified iron oxide in a glass vial and sonicated for 10 minutes. 3 ml of 8% glutaraldehyde was added in PBS containing APTES modified iron oxide and mixed for 5-6 hours at room temperature in a magnetic stirrer. Magnetic nanoparticles were separated and suspended in 4.0 ml of PBS for immobilization [6].

2.3.3 Protocol for immobilization of L-asparaginase on the MNPs :

80 µl of 4.0 ml of PBS solution containing 10 mg of glutaraldehyde activated MNPs was added to 920 µl of L-asparaginase in PBS. Mixed for 20 hrs in a magnetic stirrer and separated the MNPs with a round magnet. 1 ml of PBS was added and vortexed briefly. The suspension containing MNPs (0.20 mg) in 1.0 ml of PBS stored for further usage [6].

2.3.4 Characterization of L-asparaginase coated iron nanoparticle and Iron Nanoparticles

2.3.4.1 UV- Visible Spectroscopy

Bio reduction of FeCl$_3$ and FeCl$_2$ in the aqueous solution was monitored by periodic sampling, after diluting small aliquots (0.3 ml) of sample of millipore water. UV-Vis spectroscopy analysis of iron nanoparticles were recorded as a function of time at room temperature using UV-Vis spectrophotometer. The synthesized L- asparaginase coated iron nanoparticle characterized under Systronic Double beam spectro- photometer: 2202. The sharp bands of iron colloids were observed. The absorption peaks were observed at 200 – 600 nm. The reduction of metal ions was monitored by measuring the UV-Vis spectroscopy of the solution by the sampling of aliquots of the aqueous component. The samples were exposed to the cyanide compounds shows the wide spectrum range around 400 to 600 nm.

2.3.4.2 Fourier Transform Infra-red Spectrophotometer (FT-IR)

Fourier Transform Infrared Spectrophotometer is the most dominant analysis technologies for organic substance (solid state). When analyzing organic substance, it is very common to measure with FT-IR in the first place. The technology is utilized in analyzing foreign material. When irradiate infrared beam on substance, light beams with specific wave length are selectively absorbed; and resulting infrared absorption spectrum is inherent to independent solid-state substance, just like the finger print is inherent to individual.

2.3.4.3 Transmission Electron Microscopy

The prepared iron and L- asparaginase coated iron nanoparticle were taken for TEM analysis on carbon-coated copper TEM grids. The films on the TEM grids were allowed to stand for 2 min following which the extra solution was removed using a blotting paper and the grid was allowed to dry prior to measurement.

2.4 Anticancer Enzyme Activity :

Determination of Invitro Antiproliferative Effect of Bioconjugated Nanocomposites on Cultured Hela Cells

HeLa cells were purchased from NCCS Pune was maintained in Dulbecco’s modified eagle media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5% CO2 (NBS, EPPENDORF, GERMANY) in a humidified atmosphere in a CO2 incubator. The cells were tryspinized (500 µl of 0.025% Trypsin in PBS/0.5mM EDTA solution (Himedia) for 2 minutes and passaged to T flasks in complete aseptic conditions. 1µl, 5µl and 10 µl of sample was added to 80% confluent and incubated for 24 hours. The % difference in viability was determined by standard MTT assay after 24 hours of incubation.

2.4.1 MTT ASSAY: MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5 dimethythiazol 2-yl) -2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilised formazan product was measured at 540nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The cell culture suspension was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at 37°C for 3 hours. MTT was removed by washing with 1x PBS and 200 µl of DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank. % viability was calculated using the formula

% viability = (OD of test/OD of control) x100
3. RESULTS AND DISCUSSION
3.1 Microbial Synthesis of L-Asparaginase: The microbial strain *E. coli* was streaked on the surface of sterilized Eosin Methylene Blue agar (EMB) plates (Fig. 1. a) After 24 hr incubation, the plate showed a green metallic sheen, so it was confirmed as *E. coli* and preserved on Nutrient agar slants (Fig. 1. b).

![Plate showing a green metallic sheen on EMB agar](image1.png)

![Nutrient agar slant](image2.png)

3.1.1 Determination of L-Asparaginase Activity
3.1.1.1 Qualitative assay for screening of L-asparaginase production
The release of ammonia from asparagine in M-9 medium plate led to increase in local pH. M-9 medium plate showed colonies which have exhibited clear pink zone surrounding microbial colonies after 48 hrs (Fig. 2. a) The plate assay is advantageous as the method is quick and L-asparaginase production can be visualized directly from the plates without performing time consuming assays [3]. Czapek Dox’s Medium agar tubes was changed from yellow to pink after 48 hours of incubation. *E. coli* strain showed a positive result color intensity of the medium was increased with incubation hence the reddish appearance (due to the presence of phenol red indicator) of plates and tubes harboring positive cultures (Fig. 2. b)

![Plate showing pink colonies](image3.png)

![Tubes showed from yellow to pink colour](image4.png)

3.1.1.2 Production of L-asparaginase by submerged fermentation
In submerged fermentation the production of L-asparaginase using *E.coli*, after incubation period it showed turbidity growth on production media (Fig. 3. a and Fig. 3. b) The enzyme activity was confirmed using agar well diffusion method and the medium showed 6.2 cm zone of enzyme activity turned its colour from yellow to pink after incubation in 48 hrs (Fig. 3. c)
3.1.2 Purification of L-asparaginase: Characterization of purified L-asparaginase

**Determination of the L-asparaginase molecular weight**

The enzyme was extracted from the production media by centrifugation and further purified by ammonium sulphate precipitation and dialysis. The crude protein was collected and its molecular weight was determined using SDS PAGE. The partially purified enzyme protein was then subjected to column chromatography using Sephadex G-100. In 8 fraction were obtained and the 4th fraction showed maximum activity and its molecular weight was determined as 110 kDa (Fig. 4) Similar results were observed by purified L-asparaginase from sewage water of *E.coli* [16], pathogenic *E.coli* [5], which exhibited a molecular weight of 56 and 175 kDa respectively.

**Figure. 4. Electrophoretic analysis of L-asparaginase produced by E.coli**

**3.1.3 Quantitative Assay of L-Asparaginase**

The enzyme assay of purified L-asparaginase was determined using Nessler’s reagent. The most active 4th fraction showed enzyme activity 5.24 U/ml. [2] reported the production of L-asparaginase in submerged fermentation where in the maximum of 3.8 U/ml at 96 h of incubation period was attained. [17] reported that the highest L-asparaginase activity of *A. terreus* in liquid medium was found at 48 hr while in solid medium the optimal period for enzyme production was 96 hr. [20] indicated that the L-asparaginase production was maximum 6.05 IU at 72 hr fermentation period. This was more identical with our findings in modified M-9 broth which gave maximum enzyme activity at 48 hours. According to this work enzyme activity of L-asparaginase from *E.coli* was 5.24 U/ml (Fig. 5)

**Figure.5. Quantitative Assay of L-asparaginase activity**

3.2 Synthesis of Iron Nanoparticles by Chemical Method

The iron nanoparticles were synthesized by co precipitation method. The formation of black colour precipitate indicated the presence of iron nanoparticles. The synthesized iron nanoparticles are shown in (Fig. 6)

**Figure.6. chemical synthesis of magnetic iron oxide nanoparticles**

3.3 Iron Nano Particles Bioconjugated with L-Asparaginase

L-asparaginase was bioconjugated with iron nanoparticles using three method APTES modification of magnetic iron nanoparticles (Fig. 7. a) Glutaraldehyde activation (Fig. 7. b) and Bioconjugation of Iron Nanoparticles to Target Enzyme are shown in (Fig. 7. c)
3.4 Characterization of Synthesised Iron Nanoparticles and L-Asparaginase Bioconjugated with Iron Nanoparticles

3.4.1 UV-Visible Spectroscopy

UV-vis absorption is a simple and applicable measurement to explore the structural changes and prove the complex formation. The protein could easily adsorb onto the nano particles via electrostatic interaction. The uncoated iron nanoparticles in aqueous solution showed absorption maximum around 384.8 nm, which is attributed to the typical plasmon band of the nanoscale iron. Enzyme conjugated iron nanoparticles showed a slight shift in the surface plasmon band from 384.8 to 412.5 nm (Fig. 8 a) The absorption band was mainly due to the transition of enzymes characteristic polypeptide backbone structure. The conformational changes reflected by the spectral difference at 384.8 nm in the UV-vis spectra might arise from disturbances of polypeptide’s environment of the protein. Chemically synthesized iron nanoparticles showed absorption at 286 nm (Fig. 8 b). [11] reported the absorption maxim at 404.0 nm in visible range between 200 to 800 nm wavelengths.

3.4.2 FTIR

FTIR analysis was carried out for the chemically synthesized iron nanoparticles and a peak was observed in 1537 cm⁻¹ is attributed to the stretching vibration of C =O and carboxylate groups indicates 1363 cm⁻¹ (Fig. 9 a) FTIR spectra of L-asparaginase coated iron nanoparticles showed peak at 3748 cm⁻¹ (–OH group ) and consists the characteristic bands of L-asparaginase enzyme at 2781 cm⁻¹ (asymmetric stretching vibration of CH₂ group), 1595 cm⁻¹ (–NH group ), 1389 cm⁻¹ (C-N amine ) and 1080 cm⁻¹ amino group that represents MNP with proteins and Carboxylate groups of enzyme. The presence of enzyme on the NP surface was confirmed by FT-IR spectroscopy (Fig. 9 b) [4] reported that FTIR spectrum of magnetic particles shows deformation of two methyl groups on –CH(CH₃)₂ at 1368 and 1386 cm⁻¹, –CH₃ and –CH₂ deformation at 1460 cm⁻¹, –CH₃ symmetric stretching at 2877 cm⁻¹.

3.4.3 TEM

The transmission electron microscopy (TEM) image of the chemically synthesized iron nanoparticles indicates well dispersed particles which are more or less spherical. The average size of these particles are approximately 30 nm. The results by TEM indicate that the nanoparticles consist of agglomerates of small grains 6.58 nm (Fig. 10 a) [18] stated that the size of the magnetite particles as 50 nm carried out by co-precipitation method. [9] reported the average particle size of the pristine Fe₃O₄ MNPs was about 16.5 nm which was larger than the oleic acid modified Fe₃O₄ NPs and TEM analyzing enzyme coated MNP was at a range of 500nm (Fig. 10 b)
3.5 MTT Assay for Evaluation & Apoptosis
Using MTT assay, the in vitro bioassay cytotoxic effect of *E.coli* enzymes, iron nanoparticles and bioconjugated enzyme on the growth of normal and cancer cell lines (HeLa). However, the highest antitumor activity was recorded towards cancer cell lines than normal cell lines with increase in the concentration. Cytotoxic study shows more cell death in enzymes coated iron nanoparticles HeLa cell lines (Fig. 11. a) than normal cell lines (Fig. 11. b).

![Image](http://ijesc.org/)

**Figure. 10.** a. TEM of chemical synthesized iron nanoparticles

**Figure. 10.** b. Enzyme coated MNP

**Figure. 11.** a. Invitro analysis against normal cell lines
The result of HeLa cell lines clearly shows highest activity of L-asparaginase coated iron nanoparticles was obtained 91 %, while least activity in iron nanoparticles was obtained 47 % (Fig. 12) of viability at the minimum concentration of 1 µl, while in normal cell lines shows highest activity of L-asparaginase coated iron nanoparticles was obtained 77 %, while least activity in iron nanoparticles was obtained 32 % of viability at the minimum concentration of 1 µl, when compared to both normal and HeLa cell lines concentration of 5 and 10 µl (Fig. 13 and Fig. 14)

**Figure. 11.** b. Invitro analysis against cancerous cell lines

**Figure. 12.** a. Control

**Figure. 12.** b. cells treated with 1µl of sample

**Figure. 12.** In-vitro analysis in Cancer cell lines at different concentrations of iron nanoparticles in phase contrast microscopy

![Image](http://ijesc.org/)

http://ijesc.org/
Figure 12. c. cells treated with 5µl of sample

Figure 12. d. cells treated with 10µl of sample

Figure 13. In-vitro analysis in Cancer cell lines at different concentrations of L-asparaginase in phase contrast microscopy

Figure 13. a. Control

Figure 13. b. cells treated with 1µl of sample

Figure 13. c. cells treated with 5µl of sample

Figure 13. d. cells treated with 10 µl of sample

Figure 14. In-vitro analysis in Cancer cell lines at different concentrations of L-asparaginase coated iron in phase contrast microscopy

Figure 14. a. Control

Figure 14. b. cells treated with 1µl of sample

Figure 14. c. cells treated with 5µl of sample

Figure 14. d. cells treated with 10µl of sample
The L-asparaginase of E.coli has anti-proliferative activities so it does not affect normal cells but it only affected the cancerous cells. Similar studies showed [14] studied antitumor activity of L-asparaginase from Thermus thermophilus against different cell lines.

[1] that the anti-tumor activity of crude enzyme extracts of Penicillium brevicompactum on the growth of four human tumor cell lines, the highest activity was obtained towards Human heptocellular carcinoma cell line (65.3%), while least activity was obtained towards Human lung carcinoma (33%).

4. CONCLUSION

The enzyme L-asparaginase was produced in the medium from E.coli and metal salts Iron (anti-cancer agent) were added to the culture filtrate separately to form their respective Nanoparticles. The nanoparticles produced were found to be coated with the enzyme L-asparaginase forming their respective Metallo-proteins. The Metallo-proteins or Metal-asparaginase nano biocomposites exhibited higher Anti-Cancerous activity than the enzyme L-asparaginase alone.

The Metallo-proteins also have the advantage of using them in higher dosage levels with reduced side effects as the Metallo-protein does not precipitate in the blood which leads to the uneasy immunogenic reactions. Thus, their improved usage in Cancer Therapy. These Metallo-proteins can also be coupled along with other cancer cells targeting agents like folate to form a Nanocarrier for their specific drug delivery action in the treatment of Cancer.

5. REFERENCES


