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# ENHANCED IN VITRO CYTOTOXICITY EFFECT OF GALLIC ACID IN MCF7 CELLLINES BY USING CHITOSAN NANOPARTICLES AS A TARGET VECTOR

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## ABSTRACT
Cancer is the second leading cause of death worldwide. There is always a huge demand for novel anticancer drugs and scientists explore various natural and artificial compounds to overcome this. Gallic acid (GA) is one of the phenolic acids found in many dietary substances and herbs used in ancient medicine. It possesses antiinflammatory, antioxidant, antiviral and antibacterial properties. The main objective of this work was to prepare and characterize chitosan loaded gallic acid nanoparticles. The organoleptic propertity of the drug were complys with USP standard. The solubility studies were performed. The FTIR studies were carried out for pure drug, polymer and drug with polymer in different ratio which shows there was no chemical interaction between the drug and polymer. The drug content and entrapment efficiency of the nanoparticle was increase as the concentration of polymer increase. The F4 formulation shows more entrapment efficacy compared to other formulations nearly 70.87% and the drug content was found to be 86.7%. The particle size and the surface morphology results revealed that gallic acid nanoparticles (GNPs) were smoothed spheroidal with a size ranging from100-200nm. The invitro dissolution studies were carried out for all the Formulation. Formulation F4 shows 97.67% of drug release at the end of 24 hr. in this study we extensively investigated the anticancer effect of GA in human breast carcinoma MCF-7 cells. the result revealed that GA induce apoptosis by triggering the extrinsic or Fas/ FasL pathways.

**KEEYWORDS**: Gallic acid, MTT- assay, MCF-7 cell line, nanoparticles.

## INTRODUCTION
Cancer is one of the leading causes of death in the world. According to the 2014 World Cancer Report, approximately 14 million new cancer cases and 8.2 million cancer-related deaths were reported in 2012. The number of new cancer cases is expected to increase by 70%, from 14 million to 22 million, in the next 2 decades. The populations of Africa, Asia, and Central and South America represent 70% of all cancer deaths and 60% of the total new annual cancer cases worldwide. Several therapies are available to treat various types of cancer.[I] Chemotherapy in combination with cytotoxic agents is the most commonly utilized therapy to control many types of cancer. But In cancer therapy, the efficacy of conventional cytotoxic agents is dramatically reduced due to their unfavorable physico-chemical and pharmacological properties, consisting in poor water affinity, non-specific distribution within the body, severe toxic effects to healthy cells and tissues, inadequate drug concentrations at tumors sites, and the development of multi-drug resistance. Based on these undesirable side effects, the National Cancer Institute (USA) has encouraged the investigation of the potential antitumor activities of plant extracts. Natural compounds isolated from medicinal plants are believed to be promising leads in the development of anticancer drugs.

Medicinal plants have been used widely in anticancer studies due to their high efficacy and limited side effects. The anticancer efficacy of medicinal plants has been studied in clinical trials and has shown positive results, Since ancient times, numerous medicinal plants extracts and their active components have been reported to have potential uses as anticancer agents. Numerous studies have reported that medicinal plants display anticancer and cytotoxic activities.[2] In that Polyphenols are valuable compounds possessing scavenging properties towards radical species and are involved in defence against ultraviolet radiation or aggression by pathogens these abilities making polyphenols important for the treatment of various diseases like inflammation or cancer, but also for anti-ageing purposes in cosmetic formulations, or for nutraceutical applications.[3] Thus, the systematic use of antioxidant molecules to reduce oxidative stress seems to be the ideal approach for cancer prevention (Hou et al., 2005; Khan et al., 2006; Lambert & Elias, 2010). Consistently, extensive epidemiology evidence both in animal and in human studies, suggested that specific dietary antioxidants could be effective in

preventing cancer incidence and mortality (Dai & Mumper, 2010). A 5–7% increase in mean survival time and 25–39% decrease in tumor load was recorded in mammary adenocarcinoma bearing mice treated with 0.05% green tea polyphenols as the sole source of drinking fluid and this was correlated with reduced level of malonyldialdehyde–DNA adduct (a marker of oxidative stress) (Kaur et al., 2007).

These properties of polyphenols are also responsible for a lack in long term stability, deficiency avoided by encapsulation methods described in the literature. The reports on encapsulation of polyphenolic antioxidants by ionic gelation process are limited. Liang prepared by this method chitosan nanoparticles loaded with tea polyphenol extract. The particles have been proved to be good nanosystems for slow release, the polyphenolic material being actively maintained.

Chitosan is a polysaccharide derived from naturally occurring chitin. Its unique properties make it attractive for many industrial and biomedical applications (including controlled drug release, wound healing, nutrition supplements, water purification, removal of toxins, scaffolds for tissue engineering, and semipermeable membranes). Due to its pH dependent solubility, it forms stable films on various surfaces under neutral and basic pH conditions. Its amine groups serve for covalent attachment of biomolecules, and it can be co-deposited with other polymers or nanoparticles.

The present study involves synthesis, characterization and biological studies of nanoparticles conjugated with herbs for their use against breast cancer.

## MATERIALS AND METHODS

Gallic acid, chitosan and tripoly phosphate was obtained from Otto biochemika, acetic acid, sodium hydroxide and potassium dihydrogen phosphate were obtained from SISCO research laboratories, India. All equipments for the synthesis and evaluation were carried out in C.L. Baid metha college of pharmacy and ramachandra university.

## PREPARATION OF NANOPARTICLES CONTAINING GALLIC ACID.

A Chitosan nanoparticle was prepared by ionotropic gelation process. Chitosan solution (0.1% w/v) as prepared by dissolving 100 mg of chitosan in 100ml of 1% v/v acetic acid.TPP (tripolyphosphate) solution of 0.1% was prepared by dissolving 100mg of TPP in 100ml of deionized water. gallic acid was added to the TPP solution. The chitosan solution was then stirred at1500rpm for 30min in an ultrasonicator (vibronics) and add TPP solution containing lamivudine drop by drop (10ml) and kept stirring for 1hr on magnetic stirring. Nanoparticles were obtained upon the addition of a TPP aqueous solution to a chitosan solution.[4] The NP suspension is then centrifuged at 15,000 rpm for 10 min using high-speed centrifuge (Sigma). Discard the sediment and the preserve the supernatant. The formation of nanoparticles results in interaction between the negative groups of TPP and the positively charged amino groups of chitosan.

**Table 1: Formulation of Gallic acid Nanoparticles.**

| Sr.NO | Ingredients | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|---|
| 1 | Gallic acid | 30mg | 30mg | 30mg | 30mg | 30mg | 30mg |
| 2 | Chitosan | 0.4% | 0.2% | 0.3% | 0.5% | 0.25% | 0.35% |
| 3 | Tripoly phosphate | 40ml | 40ml | 40ml | 40ml | 40ml | 40ml |
| 4 | 0.1% Acetic acid solution | 100ml | 100ml | 100ml | 100ml | 100ml | 100ml |

## EVALUATION STUDIES

### Preformulation studies

### Organoleptic properties

Color, odor, taste, and appearance play an important role in the identification of the sample and hence they were recorded in a descriptive terminology. The result was shown in table 2.

### Solubility studies

The solubility of drug and polymer was carried out in various solvents such as distilled water, buffer solutions and organic solvents. The resulting solutions were filtered and analyzed for dissolved drug by measuring absorbance at 270 nm.

### Compatability studies*:* Fourier Transform InfraRed Spectroscopy(FTIR) studies

The fourier transform infrared ana lysis was conducted for the structure characterization. FTIR spectra of gallic acid, pure polymer and formulated nanoparticles were recorded.

FTIR spectra were recorded on Bomem FTIR MB II Spectrophotometer. Test samples were mixed with KBr, pressed into a pellet and scanned from 400 to 4000 $cm^{-1}$. The result was shown in Fig 1.

### Characterization of nanoparticles

The optimized nanoparticles containing gallic acid were characterized by studying various physico-chemical properties.

### Particle size

Nanoparticle size was determined using particle size analyzer (Germany). All samples were diluted with ultra-purified water and the analysis was performed at a scattering angle of 90° and at a temperature of 25°C.[5] The mean diameter for each sample and mean

hydrodynamic diameter was generated by cumulative analysis in triplicate. The result was shown in Fig 2.

**Drug content**

The total drug amount in nanosuspension was determined spectrophotomertically. A 0.50-ml aliquot of nanosuspension was evaporated to dryness under reduced pressure at 35°c.[4] the residue was dissolved in water and filtered with a 0.45µm filter, and gallic acid content was assayed spectrophotomertically at 270nm. The result was shown in table 3.

$$Total\ Drug\ Conent = \frac{Volume\ total}{Volume\ aliquot} \times drug\ amount\ in\ aliquot$$

**Drug entrapment efficiency**

The entrapment efficiency is also known as Association Efficiency.[6] The drug loaded nanoparticles are centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer. Entrapment efficiency was calculated as follows: The result was shown in table 3.

$$DEE\% = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

***In- vitro* release studies**

In-vitro diffusion studies (drug release studies) were performed by using diffusion apparatus .A semi-permeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was phased towards donar compartment. The glass beaker was filled with 100ml of phosphate buffer of (ph:6.8) at a temp 37°c sample of 1ml was withdrawn at regular intervals from glass beaker for analysis.1ml of phosphate buffer was replaced immediately after sampling to maintain volume equal to 100ml.[7] The absorbance of sampling was measured at 270nm for gallic acid and 266 nm for 5-fluorouracil by using uv spectrophotometer.[8] The result was shown in Fig 3.

**DPPH radical scavenging activity**

About 0.1M solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of the different concentration (25-800 µg/ml) of chloroform fraction (CF) and control in different test tubes.[9] The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 270 nm using a spectrophotometer.

% Inhibition= (Abs Control – Abs Sample) x 100/ Abs Control Where Abs Control is absorbance of control at time = 0 and Abs Sample is absorbance of test sample. The IC50 Value for extracts was also calculated. The results are mentioned in Figure 4 and table 5 of results and discussion portion.

**Cytotoxicity studies –MTT ASSAY**

MTT (3- 4, 5-dimethyl thiazol-2yl)-2,5-diphenyltetrazoliumbromide were cleaved by mitochondria dehydrogenase in viable cells there by yield a measurable purple product Formosan. This Formosan production is proportionate to the viable cell number andinversely proportional to the degree of cytotoxicity.

**Assay procedure:** After incubation, remove the medium from the wells for MTT assay. And add 200µl of MTT concentration of (5mg/ml and incubate for 6-7hrs in 5% CO2 incubator. After incubation 1ml of DMSO was added in each well and mix by pipette and leave for 45seconds and it shows the purple color formation.[12] The suspension is transferred in to the cuvette of spectrophotometer and O.D values are read at 270nm and % of cell viability was calculated using the formula. The result was shown in Fig 7.

Graph was plotted using the % of cell viability at Y-axis and concentration of the sample in X-axis.

(OD of sample/OD of cell control) × 100 = % cell viability

**Apoptosis Analysis by Flow Cytometry**

To investigate the apoptotic effect of gallic acid nanopaticles on MCF-7 cells, these drugs were tested by using Annexin V- FITC Detection Kit. 1x105 cells/well 33 were seeded in a 6-well plate in 1,98 mL growth medium and incubated at 37°C in 5% CO2 for 24h. After incubation, 20 µLgallic acid nanoprticles were added to the concentration of drug ranging from 100, 300, 500 and 1000.0 mL for MCF-7 cell lines. Then, these treated cells were continoually fostered in the medium at 37°C with 5% CO2 for 72h. Untreated cells were used as control groups. After incubation, the cells were taken to a falcon tube and centrifugated at 800 rpm for 5 minutes. When centrifugation finished, the pellet was dissolved in 5 mL of PBS and centrifuged again. After that, the pellet was resuspended in 200 µL of binding buffer. 2 µL of Annexin V-FITC and PI were added.[12]The stained cells were incubated for 15 minutes at room temperature. Finally, the mixture was determined by flow cytometer . the result was shown in Fig 8.

**Scanning electron microscopic (SEM) analysis**

The morphology characteristics of the prepared nanoparticles were examined under the scanning electron microscope (JSM-6360LV Scanning Microscope; Jeol, Tokyo, Japan). Before microscopy, nanoparticles were suspended in a phosphate buffer (pH 7) by vortex for 1 minute, and then one drop was spread on a small clean slide cover and left to dry overnight in a desicator. In the next day, they were mounted on carbon tape and sputter-coated using a thin gold palladium layer under an argon atmosphere using a gold sputter module in a high-vacuum evaporator (JFC-1100 fine coat ion sputter; Jeol, Tokyo, Japan). The coated samples were then scanned

and photomicrographs were taken at an acceleration voltage of 10 kV. The result was shown in fig 9.

## RESULT AND DISCUSSION

### Preformulation studies

All the organoleptic characters of gallic acid and 5-fluorouracil were studied and it was found that all the characters comply with USP standards.

**Table 2: Organoleptic properties of gallic acid.**

| Properties | Results |
|---|---|
| Description | Crystalline |
| Taste | Slightly bitter |
| Odour | Odourless |
| Colour | White to off white crystalline solid |

### Solubility

The drug was found to be very soluble in water, pH6.8 phosphate buffer, 1M HCL and pH4.5 acetate buffer.

### Compatibility studies*:* (FTIR)

The FTIR spectrum of gallic acid, polymer and the prepared nanoparticles were studied to detect the compatibility studies. The peaks in the IR spectrum of gallic acid were compared with that of the prepared nanoparticles. The distinct peaks at 1722.67 shows a C=O stretching and peaks at 1646, 1591 and 1502 shows at N-H bending. From the above observations we can conclude that, there is no incompatibility between the pure drug and polymer.

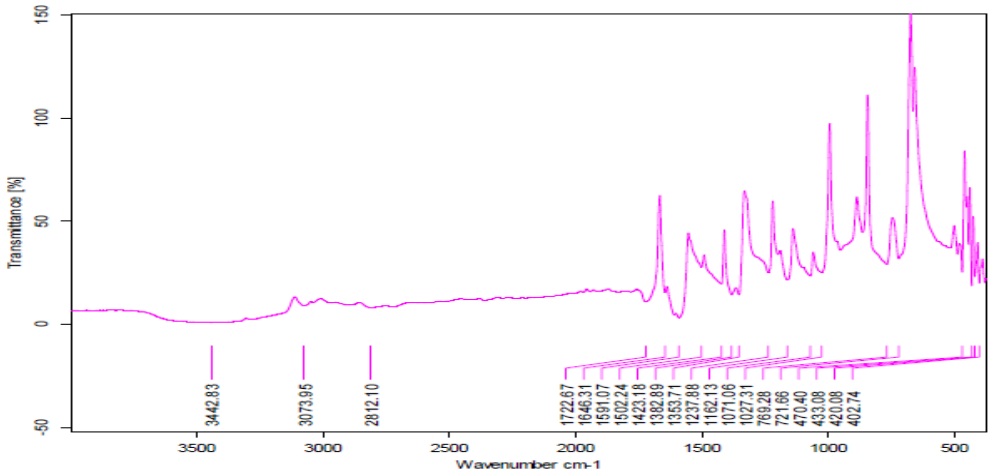



**Fig. 1: FTIR spectrum of gallic acid and chitosan.**

### Measurement of particle size of Nanoparticles

The particle sizes of prepared nanoparticles were measured from the microphotograph of 100 particles. The particle size ranged from 138.1to 165.4 nm for various batches.

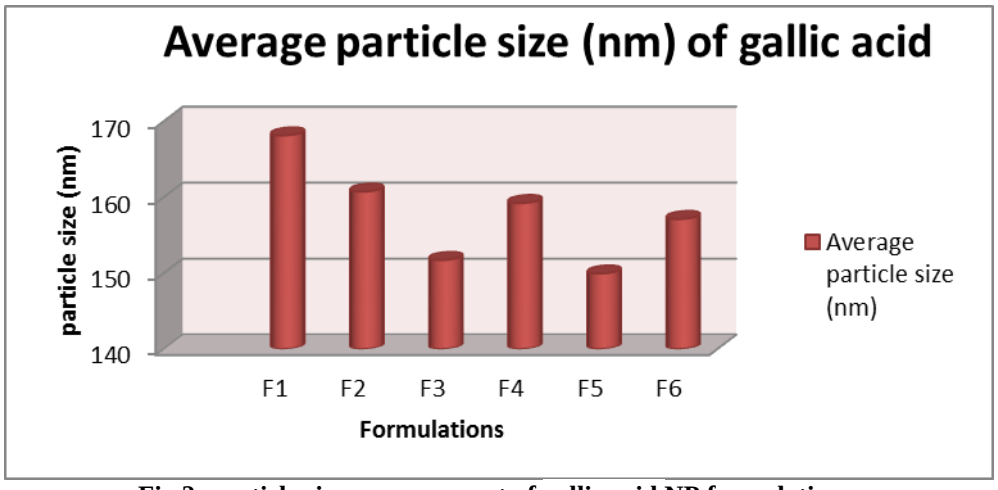



**Fig 2: particle size measurement of gallic acid NP formulations.**

### Drug content and entrapment efficiency

The total drug amount in nanosuspension was determined spectrophotomertically. A 0.50-ml aliquot of nanosuspension was evaporated to dryness under reduced pressure at 35° c. the residue was dissolved in water and filtered with a 0.45µm filter, and Gallic acid content was assayed spectrophotomertically at 270nm.

**Table 3: Drug content and entrapment efficiency of Gallic acid nanoparticle formulations**

| Formulation | Average entrapment efficiency | Drug content |
|---|---|---|
| F1 | 57.1% | 65.4 |
| F2 | 65.26% | 56.8 |
| F3 | 65.14% | 80.9 |
| F4 | 71.87% | 89.7 |
| F5 | 61.41% | 70.4 |
| F6 | 57.28% | 82.7 |

***In vitro* release studies**

In vitro release studies In vitro diffusion studies (drug release studies) were performed by using diffusion apparatus .A semi permeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was placed towards donor compartment.

The formulation F1 shows 79.9% of drug release at the end of 24 hr The formulation F2 shows 90.1% of drug release at the end of 24 hr.

The formulation F3 shows 84.06% of drug release at the end of 24 hr. The formulation F4 shows 96.9% of drug release at the end of 24 hr compared to other formulation.

The rate of release was based on concentration of a polymer, if the concentration of polymer increases the rate of release of a drug was slow due to high bio-adhesive character of polymer. Based on the solubility nature of an polymer the drug will get release slowly in a desire amount at the particular site of region there by fluctuation of drug was avoided and the plasma concentration of an drug was maintained there by the therapeutic action of an drug was more at the site of action so the side effects were avoided.

The formulation F5 and F6 shows 90 % of drug release at the end of 24 hrs. There by the sustained action of a drug at the targeted site was obtained. The result was shown in table no.

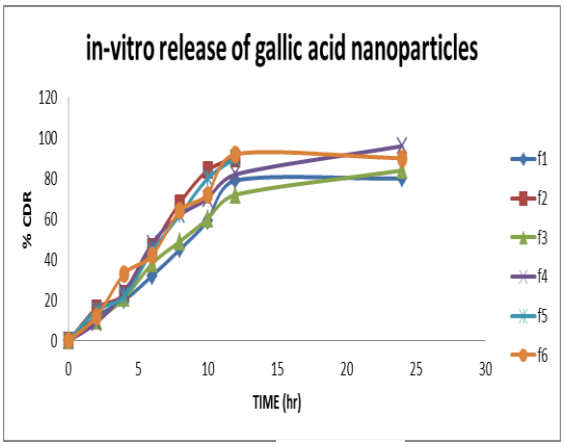



**Fig 3: *In-vitro* release of Gallic acid nanoparticles.**

The polymer chitosan is soluble in acidic solutions owing to the protonation of amine group in the polymeric chain and readily soluble in solution with a PH upto 6.5. In tumor cells ph values are low (ph 5.5) due to high anaerobic glucose metabolism. The drug release rate can be rapidly accelerated with the low ph of the tumor cells. Chitosan nanoparticles have been demonstrated to be PH- responsive system with reversible process of swelling and shrinkage of particles.

In the present study chitosan nanoparticles showed the effective drug release pattern in the tumor environment at the same time it has an intrinsic antitumor activity.

In the present study the reason for choosen the polymer in different concentration reveals, higher the concentration of polymer will increase the mucoadhesive characters of an polymer thereby the release of drug will based on solubility of polymer in different pH range, higher the concentration of polymer will give sustained release of drug to achieve the therapeutic activity of drug thereby wastage of drug and side of drug can be avoided.

**Kinetic modelling**

The release kinetics was studied by fitting the values in zero order, first order, Higuchi equation and Korsmeyer-Peppas equation. The equation of best fit was determined. The values showed that the release follows first order ($R^2$ = 0.9929), ($R^2$ = 0.9497 a) Korsmeyer peppas equation and does not follow zero order and Higuchi equation. The "n" value is 0.78 hence the drug release follows anomalous Non-Fickian Diffusion. The result was shown in table no 4.

**Table 4: *In-vitro* release kinetics of optimized formulation of gallic acid nanoparticle.**

| Sr..No | Release kinetics | $R^2$ |
|---|---|---|
| 1 | zero order | 0.7893 |
| 2 | First order | 0.9929 |
| 3 | Higuchi equation | 0.8905 |
| 4 | Korsmeyer Peppas equation | 0.9534 |

**DPPH ASSAY**

**Table 5: DPPH radical scavenging activity of gallic acid.**

| Conc. | Ascorbic acid | Gallic acid |
|---|---|---|
| 100µg/ml | 20.297±0.014 | 35.125±0.025 |
| 200 µg/ml | 32.657±0.313 | 38.249±0.115 |
| 400 µg/ml | 47.403±0.253 | 45.157±0.254 |
| 600 µg/ml | 57.052±0.668 | 58.842±0.025 |
| 800 µg/ml | 72.0840±0.790 | 74.265±0.458 |
| 1000 µg/ml | 78.261±0.526 | 79.157±0.165 |
| $IC_{50}$ Value | 510(µg/ml) | 485(µg/ml) |

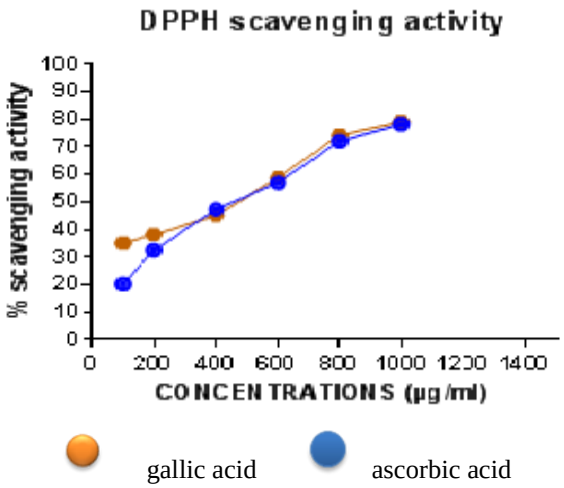



**Fig 4: DPPH scavenging activity of Gallic acid.**

In the DPPH radical scavenging assay DPPH radical was used as a substrate to evaluate free radical scavenging activity of gallic acid. This involves the reaction between the a specific antioxidant and the 2,2 diphenyl picryylhydrazide (DPPH). As a result there is a reduction of DPPH concentration by the antioxidant principle present in the gallic acid which was confirmed by optical asorbance of DPPH; this was detected by spectrophotometer at 270nm. Ascorbic acid was used as a standard. The $IC_{50}$ value was found to be 510 µg/ml and 485 µg/ml for gallic acid and ascorbic acid respectively.

**MTT- ASSAY**

New cytotoxic drugs have made considerable progress in the treatment but side effects such as heart and kidney problems, suppression of the immune and hematopoietic systems are still the important causes of mortality.

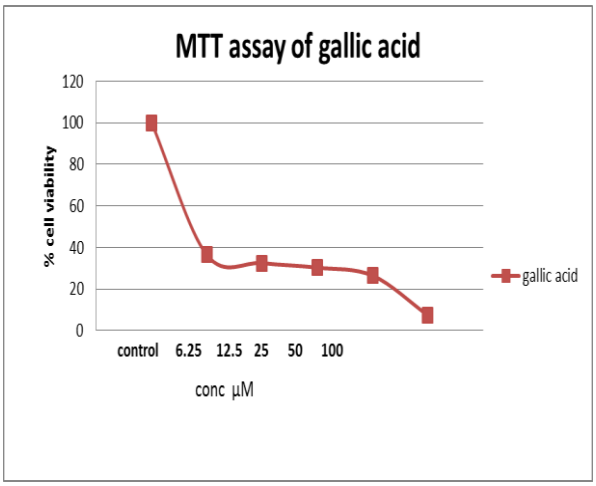



**Fig 5: Effect of gallic acid raw drug on MCF-7 cell line.**

To investigate the effect of gallic acid on MCF-7 cells, we treated MCF-7 human breast carcinoma cells with various concentrations of polyphenols for 24hr.

The study revealed , MCF-7 cells treated with 6.25, 12.5 and 25 µM of gallic acid showed after 24h significant decrease in cell viability after 24 h was found to be 36.34%, 32.48% and 30.28% respectively. While, treatment of cells with 50 and 100 µM of gallic acid caused significant decrease in MCF-7 cell viability to 26.41% and 8.28% respectively.

These data showed a gradual increase of dose decreased the percentage of cell viability thereby the drug Gallic acid has prominent inhibitory effects on human breast cancer cell proliferation, which has been determined by MTT viability assay.

The percentage of cell viability was not compatabile with the standard drug 5-fluro-uracil which may be due to physio-chemical characteristics of an drug such as solubility, short –half life, wide distribution and less bioavailability of an drug, thereby limits the medical applicability of an drug also concentration of drug at the site of action will also low. To overcome the above mentioned limitation the studies has been carried out on sustained drug delivery system using polymer like chitosan.

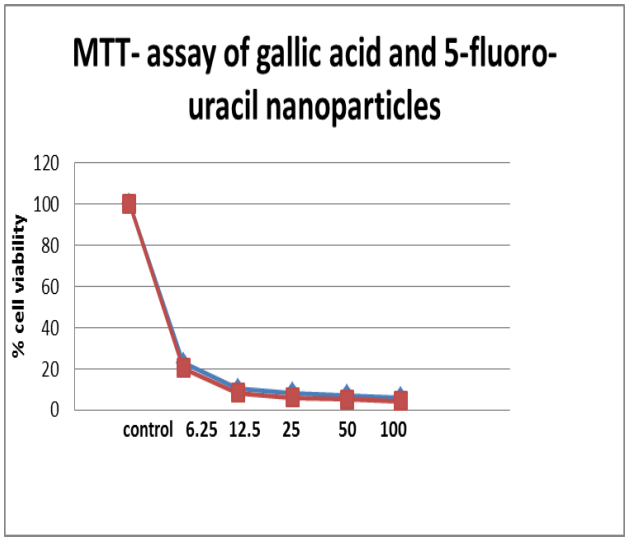



**Fig 6: *In vitro* assay for Cytotoxicity activity of gallic acid chitosan loaded nanoparticles and 5-fluoro-uracil chitosan loaded nanoparticles.**

The results of our study showed that gallicacid nanoparticles in a dose and time dependent manner increased the cell arrest by increased the induction of apoptosis in MCF-7 cells.

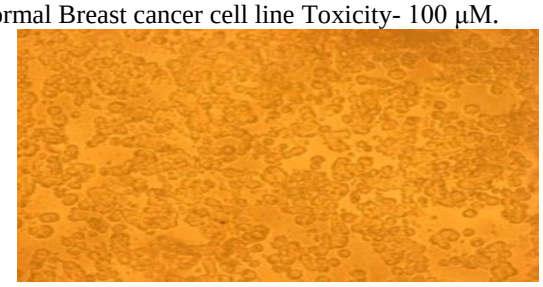
Treatment of MCF-7 cells with 6.25 µM of gallic acid loaded chitosan nanoparticles significantly decreased cell viability to 22.97% whereas cells treated with 12.5 and 25 µM of gallic acid loaded chitosan nanoparticles showed significant decrease in cell viability to 9.29% and 7.83% respectively. Likewise, treatment of MCF-7 cells with 50 and 100 µM of gallic acid loaded chitosan nanoparticles produced significant decrease in MCF-7 cell viability to 6.19% and 5.94% respectively

Treatment of MCF-7 cells with 6.25 µM of 5-fluoro-uracil loaded chitosan nanoparticles significantly decreased cell viability to 21.67% whereas cells treated with 12.5 and 25 µM of 5-fluoro-uracil loaded chitosan nanoparticles showed significant decrease in cell viability to 8.29% and 7.13% respectively. Likewise, treatment of MCF-7 cells with 50 and 100 µM of gallic acid produced significant decrease in MCF-7 cell viability to 5.29% and 4.93% respectively

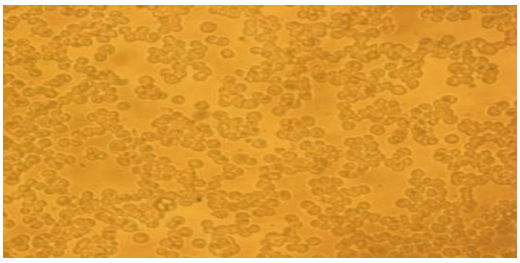
From the above study it revealed the gallic acid loaded chitosan nanoparticles showed 5.94% of cell viability at a conc of 100 µM and 5-fluoro-uracil loaded chitosan nanoparticles showed 4.93. compared to raw drug the gallic acid loaded chitosan nanoparticles showed reduced cell viability, which means compare to raw drug the formulation has high penetration efficiency and targeted the site of action thereby the bioavailabilty of an drug was increased at the site of action so dose fluctuation and over dosing of drug can be avoided

These study revealed gallic acid loaded chitosan nanoparticles has anticancer activity and was compatible with the 5-fluoro uracil nanoparticles

Normal Breast cancer cell line Toxicity- 100 µM.



**( A)**



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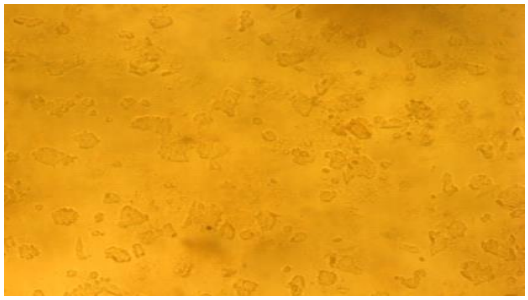

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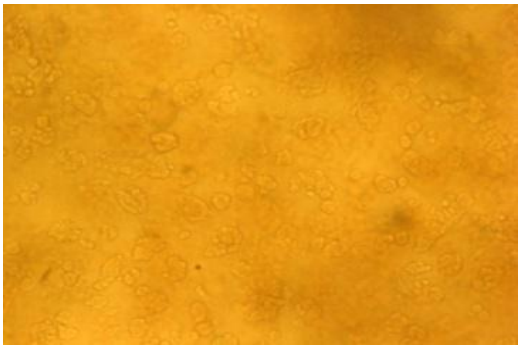

**(D)**

**Fig .7 (A) normal breast cancer cell line, (B) gallic acid raw drug (C) gallic acid nanoparticles (D) 5-fluro-uracil nanoparticles.**

In the case of the MCF-7 cell line, it was seen that the ratio of cells arrested was higher on nanoparticle treatment compared with the arrest caused by the same concentration of free drug Similarly, MCF7 cells showed a significant arrest on treatment with nanoparticles (54% and 90%), which was considerably higher than the arrest caused by free gallic acid (20.6%)

From the study it revealed the polyphenols has anticancer activity which was studied by MTT assay using MCF-7 cell line.

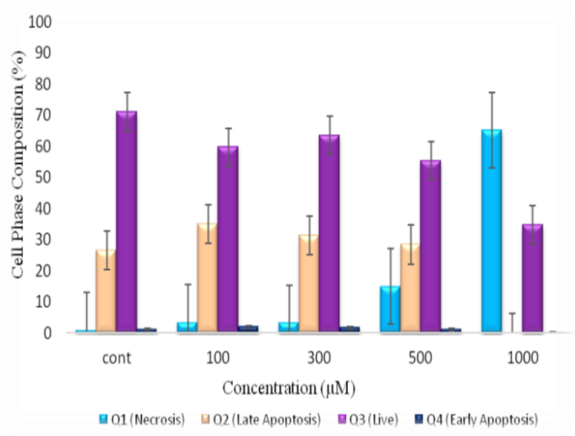


**Fig 8: Quantification of the apoptotic effects of gallic acid nanoparticles against MCF-7 cells.**

The apoptotic effects of gallic acid chitosan loaded nanoparticles against MCF-7 cells was shown in Figure 3.33. According to this result, when cell phase composition of control cells was compared with OLE-CS-NPs treated cells it was observed that there was an decrease at the amount of living cells (Q3 phase) with the increasing concentrations. Also, going through necrosis phase (Q1 phase which is a form of cell injury that results in the premature death of cells in living tissue, (Proskuryakov, Konoplyannikov, & Gabai, 2003) was observed, too. Decreasing at the amount of living cells was fit with the results of MTT assay as expected. At lowest concentrations, amount of living cells and apoptotic cells were nearly similar with the amount of control cells.

**SEM**

The size of the nanoparticles were circular and uniform

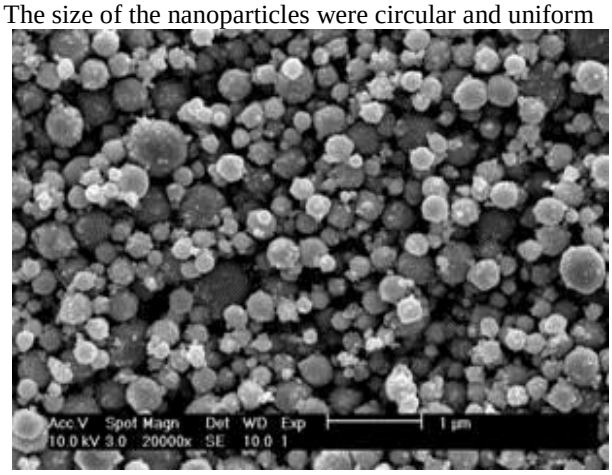


**Fig 9: Gallic acid nanoparticles.**

## CONCLUSION

In this study, we extensively investigated the anticancer effect of GA in human breast carcinoma MCF-7 cells. Because cancer cells may grow and divide more rapidly than normal cells, many anticancer drugs are made to kill growing cells. But certain normal, healthy cells also multiply quickly and chemotherapy can affect these cells, too. This damage to normal cells causes side effects. The fast-growing, normal cells most likely to be affected are blood cells forming in the bone marrow and cells in the digestive tract (mouth, stomach, intestines, esophagus), reproductive system (sexual organs), and hair follicles. Some anticancer drugs may affect cells of vital organs, such as the heart, kidney, bladder, lungs, and nervous system.

Our study indicated that treatment with GA resulted in inhibition of proliferation and induction of apoptosis in MCF-7 cells. Then, the molecular mechanism of GA's apoptotic action in MCF-7 cells was further investigated. The results revealed that GA induced apoptosis by triggering the extrinsic or Fas/FasL pathway as well as the intrinsic or mitochondrial pathway. Furthermore, the apoptotic signaling induced by GA was amplified by cross-link between the two pathways. Taken together, our findings may be useful for understanding the mechanism of action of GA on breast cancer cells and provide new insights into the possible application of such compound and its derivatives in breast cancer therapy.

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## REFERANCE

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