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Research Article

In vitro anticancer activity of silver and gold nanoparticle synthesized from *Zingiber officinale* Roscoe fresh and dried rhizomes against cancer cell lines

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ABSTRACT

Objective: The aim of the evaluation was to screen the silver and gold nanoparticle synthesized from the methanol concentrate of *Zingiber officinale* Roscoe [Family: Zingiberaceae] for its *in vitro* anticancer activity against MCF 2, PC3, A-549, HeLa and HepG2 Cell lines. **Methods:** Silver and gold nanoparticles were prepared from methanol concentrate of *Z.officinale* fresh and dried rhizomes and nanoparticle synthesised was analysed by UV, SEM, SEM with EDAX and TEM analysis. The impact of nanoparticles synthesized on MCF-2, PC3, A-549, HeLa and HepG2 disease cell lines were assessed by MTT colorimetric assay. After assessing the cytotoxicity effect, the impact of cell cycle and apoptosis were assessed by flow cytometric method. **Results:** SEM analysis showed that the morphology of nanoparticle synthesised were different from each other. TEM analysis showed the particle size of 4.11 and 28.6 nm and 54.78 and 19.25 nm for fresh and dried rhizomes synthesized silver and gold nanoparticles respectively. The adequacy of silver nanoparticle synthesised from fresh and dried rhizomes of *Z.officinale* against PC-3, MCF-2, HepG2, HeLa and A-549 cell line demonstrated that the hatching of malignancy cells decreased the cytotoxicity in cancer cells lines with IC_{50} values between 74.26 to 131.1 and 77.78 to 104.3 µg/ml respectively. Gold nanoparticles synthesized from fresh rhizomes were effective only against A-549 and dried rhizomes synthesized gold nanoparticles were effective against all except PC-3 cell lines. Cell cycle analysis and apoptosis study showed that Silver and gold nanoparticles are effective in controlling the cell cycle and blocking the apoptosis in all the cell lines used. **Conclusion:** *Zingiber officinale* can be very good anticancer drug for various cancer cell lines. All in all, *Zingiber officinale* has critical cell reinforcement movement and anticancer action.

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INTRODUCTION:

Discovery of drugs to cure cancer related disorders is continuous and persistent. Today the entire world is looking eagerly at cost effective medicines. Since large portion of the population is suffering from poverty and is looking for cheaper plant based medicines which can control cancer as the treatment cost for cancer is very costly. Nanotechnology will reduce the cost[1] as well as the toxicity of the conventional treatment. Global cancer statistics exposed the load of cancer increasing day by day. This may be due to lack of specific treatment strategies. Conventional therapies are not able to intrude with many cancers. This may be due to inadequate drug distribution pattern, high pay load, nonspecific delivery and side effects. Plants have a huge number of a functioning constituent with remedial properties. Against malignant growth capability of herbs may emerge from various systems including cancer prevention agent, mitigating, DNA fix, and acceptance of apoptosis, invulnerable initiation and hindrance of cell cycle movement[2,3.] Hindrance of cell cycle movement, relocation, and attack, together with activating apoptosis can be viewed as a technique for disease treatment.

Ginger (*Zingiber officinale* Roscoe) belonging to the family Zingiberaceae is a perennial herb. It is widely distributed in tropical Asia. In India, it is cultivated mainly in Kerala, Andhra Pradesh, Uttar Pradesh, West Bengal and Maharashtra. It is one of the most common spices, which is in use since centuries for its versatile medicinal actions like antiemetic, stomachic, expectorant, anti-inflammatory, aphrodisiac etc. in traditional system of medicine (Unani, Ayurveda, and Chinese medicine). It is useful for the treatment of various gastrointestinal, pulmonary, cardiovascular and sexual disorders. The phytochemical study of ginger showed the presence of many volatile oils and oleo-resins like gingerol, zingerone, zingiberol etc. Numerous experimental and clinical trials have proven ginger for its range of therapeutic activities such as antibacterial, anticancer, antidiabetic, antiemetic, hypolipidaemic, hepatoprotective etc properties.

The reported pharmacological actions of zinger are due presence of active chemical constituents which are 10-Gingerdione–anti-inflammatory; 6-Gingerdione–anti- inflammatory; Gingerenone-A-anticoccidioid 10 ppm; Fungicide 10 ppm; Gingerenone-B- fungicide; Gingerenone-C-fungicide; Gingerol-analgesic; Zingiberone-antimutagenic; antiulcer. 6-Gingerol, 6-and 10-dehydrogingerdione, 6-and 10-gingerdione have been reported to be potent inhibitors of prostaglandin biosynthesis (PG synthetase) *in vitro*, with the latter four compounds stated to be more potent than indomethacin. Concentration-dependent inhibition of platelet aggregation, *in vitro*, induced by ADP, adrenaline, collagen and arachidonic acid has been described for an aqueous ginger extract. Ginger was also found to reduce platelet synthesis of prostaglandin-endoperoxides, thromboxane and prostaglandins[4, 5].

Accordingly, in the present study, we green synthesised silver and gold nanoparticles from fresh and dried rhizomes and furthermore assessed *in vitro* anticancer movement against MCF-2, PC3, A-549, HeLa and HepG2 cell lines, cell cycle impact and apoptosis.

MATERIALS AND METHODS

Fresh rhizomes of *Zingiber officinale.*, 3 kg were collected from local market of Bangalore. A Voucher specimen no. TOCOP/03/2017-18 – *Zingiber officinale*] were deposited in The Oxford College of Pharmacy, Bangalore. Rhizomes were washed free of sand and 1.5 kg rhizomes were cut into small pieces and dried in sun and powdered.

Preparation of Extract

Fresh and dried rhizomes of *Z.officinale* were refluxed each with 50% Methanol separately for 30 minutes and filtered through whatman No 1 filter paper and evaporated to dryness. The percentage yield was found to be 1 % and 1.5 % for fresh and dried rhizomes of *Z.officinale* respectively. 0.1 gm each was dissolved and the volume was made up to 100 ml with methanol. These solutions are kept ready for further use at 2-8°C.

Drugs and Chemicals

Silver nitrate, Gold chloride was purchased from SD Fine chemicals Ltd, Mumbai. Propidium iodide: Cat # P4864, Sigma, RNase A: Cat # 109169, Bochringer Mannhein GmbH was

purchased. All chemicals and reagents used in this study were at least of analytical grade.

Synthesis of Nanoparticles

A set of 1 mM, 2 mM and 3 mM aqueous solution of silver nitrate and gold chloride were prepared for synthesis for silver and gold nanoparticles[6]. Exactly 9 mL of each 1 mM, 2 mM and 3 mM silver nitrate and gold chloride solution respectively was added to 0. 1 mL; 0.2 mL; 0.3 mL; 0.4 mL and 0.5 mL methanol extract of the fresh and dried rhizomes of *Z.officinale* to obtain silver and gold nanoparticles. The different concentrations of silver nitrate, gold chloride and extracts were used to standardize the optimum concentration of silver nitrate, gold chloride and extract needed for synthesis of silver and gold nanoparticles. We found 1.0 ml and 1 mM solutions were found to give best yield. The nanoparticles were synthesized at room temperature and formation of nanoparticles was confirmed by checking λmax using UV spectrophotscopy.

Lyophilisation Procedure for the Reluctant Sample Mixture

After the desired reaction period, the solution containing silver and gold nanoparticles were lyophilized. The reluctant samples were centrifuged 10,000 rpm for 15 minutes. After 15 minutes, discard the supernatant and collected the pellet and freeze dried. The lyophilized samples were kept in the freezer at 4 °C for further analysis.

Characterization of Silver and Gold Nanoparticles

Ultra Violet visible spectroscopy analyses were carried out by UV–visible spectrophotometer Shimadzu in the range of 200 nm – 800 nm, with the scanning speed of 100 nm/min. The morphology examination of dried powder samples were analysed by SEM, SEM with EDAX TEM analysis to know the morphology, particle size and amount of silver and gold ions in the prepared nanoparticles.

In vitro anticancer activity

Preparation of test solutions

32 mg/ml sample stock was prepared in sterile DMSO. For cytotoxicity studies, serial two fold dilutions from 320µg/mL to 10µg/mL were prepared which is then used for treatment.

Cell lines and culture medium

All the cell lines were procured from ATCC, stock cells was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 50,000 cells /well of was seeded in a 96 well plate and incubated for 24 hrs at 37ºC, 5 % CO_2 incubator.The Cytotoxic Assay screening was performed using MTT assay as per Mosmann[7] method to test the cytotoxicity of *Z.officinale* synthesised silver and Gold nanoparticles against MCF 2, PC3, A-549, HeLa and HepG2 cell lines. In brief, the monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37ºC for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37º C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for cell lines.

- **% Inhibition** = 100 – (OD of sample/OD of Control) x 100.

Cell Cycle Analysis

Silver nanoparticle induced changes in cell cycle

were measured using the protocol[8]. In brief, MCF 2, PC3, A-549, HeLa and HepG2 cell lines were exposed for 24 h at 10–50 µg/ml PGDSN. After the treatment, cells were fixed in chilled 70% ethanol for 1 h. Then, cells were washed twice by centrifugation, and cells were stained with propidium iodide for 60 min in dark. Stained cells were acquired by flow cytometer.

In brief, 1 x10^6 cells were seeded and cultured for 24hrs in a 6-well plate containing 2 ml of serum free media. Cells were then treated with desired concentrations of given samples were prepared in complete media and incubated for another 24hrs. Cells were then harvested and centrifuged at 2000 rpm for 5 minutes at room temperature and supernatant was discarded

carefully retaining the cell pellet. Cell pellet was washed by resuspending in 2mL of 1XPBS. The washing was repeated another time with the same conditions. Supernatant was discarded retaining the pellet. Cells were fixed by resuspending in 300 µl of Sheath fluid followed by addition of 1mL of chilled 70% EtOH drop by drop with continuous gentle shaking and another 1mL of chilled 70% EtOH added at once. The cells were then stored at 4 °C for or overnight. Post fixing, the cells were centrifuged at 2000rpm for 5mins. The cell pellet was washed twice with 2 ml of cold 1XPBS. Cell pellet was then resuspended in 500µl of sheath fluid containing 0.05 mg/ml PI and 0.05 mg/ml RNaseA and incubated for 15mins in dark. The percentage of cells in various stages of cell cycle in compounds treated and un-treated populations were determined using FACS Caliber (BD Biosciences, San Jose, CA).

Apoptosis assay

Apoptosis/necrosis induced by silver nanoparticle in cancer cell lines was analysed using Annexin-V and 7- AAD Kit (Beckman Coulter). The amount of apoptosis/necrosis in the treated MCF 2, PC3, A-549, HeLa and HepG2 cell lines was analysed by flow cytometry following the protocol[9]

In brief, The day before induction of apoptosis, plated 1 X 10^6 MCF-2, PC3, A-549, HeLa and HepG2 cell lines per well for a 6-well plate using DMEM media with 10% FBS and 1% PenStrep, incubated overnight at 37˚C at 5% CO_2.The media was replaced with test solutions of different concentrations in the media containing 10% FBS.The treated cells were incubated for 24hrs at normal culture conditions. The cells were harvested and well contents were completely transferred to the sterile FACS tubes. The cell contents were centrifuged at 2000 rpm for 5mins and supernatant was discarded. Washed the cells twice with cold PBS following the centrifugation and then resuspended the cells in 1 mL 1X Binding Buffer at a concentration of ~1 x 10^6 cells/mL. Transfer 500 µL of the cell suspension (~5 x 10^5 cells) to a new FACS tube. 5 µL Annexin V and 10 µL PI was added to the tubes, cells were gently mixed and incubated for 20 minutes at RT in the dark. The cells were analysed by flow cytometry as soon as possible (within 1 hour).

RESULTS AND DISCUSSION

The UV showed λmax at 480 nm and 560 nm respectively for Silver and gold nanoparticle formation. SEM analysis showed that the morphology of nanoparticle synthesised from fresh and dried rhizomes were different [Fig 1a, b; Fig 2a, b]. TEM analysis showed the particle size of 4.11 and 28.6 nm and 54.78 and 19.25 nm for fresh and dried rhizomes synthesized silver and gold nanoparticles respectively. [Fig 3a, b; Fig 4 a, b]. SEM EDAX showed the concentration of silver and gold ions in the prepared silver and gold nanoparticles [Fig 5 to Fig 8].

Traditionally, the *in vitro* determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by

cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of effects caused by the test material[8].Gold nanoparticles synthesized from *Z.officinale* dried sample and fresh samples showed cytotoxicity effect with IC 50 value as NA,202.9;294.4; 245.6; 245.3 and 117.4;131.1;102.2;97.83 and 74.26 µg/ml for PC-3; MCF-2; HepG2; HeLa and A549 cancer cell lines respectively.

The adequacy of silver nanoparticle synthesized against PC3, MCF-2, HepG2, HeLa and A- 549 cell line demonstrated that the hatching of malignancy cells decreased the suitability of cancer cell lines with IC_{50} values as 77.78;79.05;78.58;104.3;97.31 and 117.4;131.1;102.2; 97.83;74.26 µg/ml respectively for dried and fresh rhizomes of *Z.officinale* [Fig 9 to Fig 13; Table 1].

Fig -1a & b : SEM photos of silver nanoparticle synthesized from fresh[a] and dried [b] rhizomes of Z.officinale

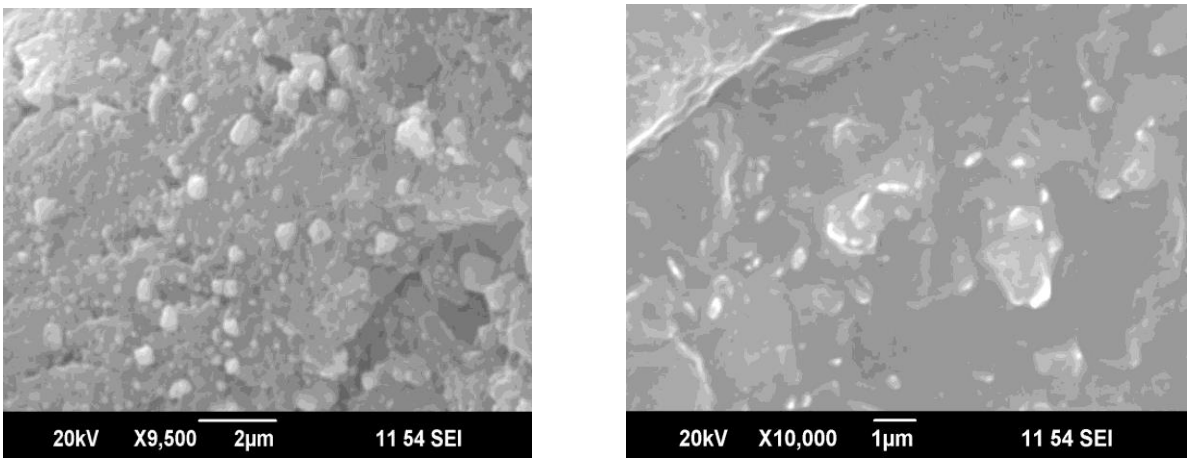


Fig 2a & b : SEM photos of gold nanoparticle synthesized from fresh[a] and dried [b] rhizomes of Z.officinale

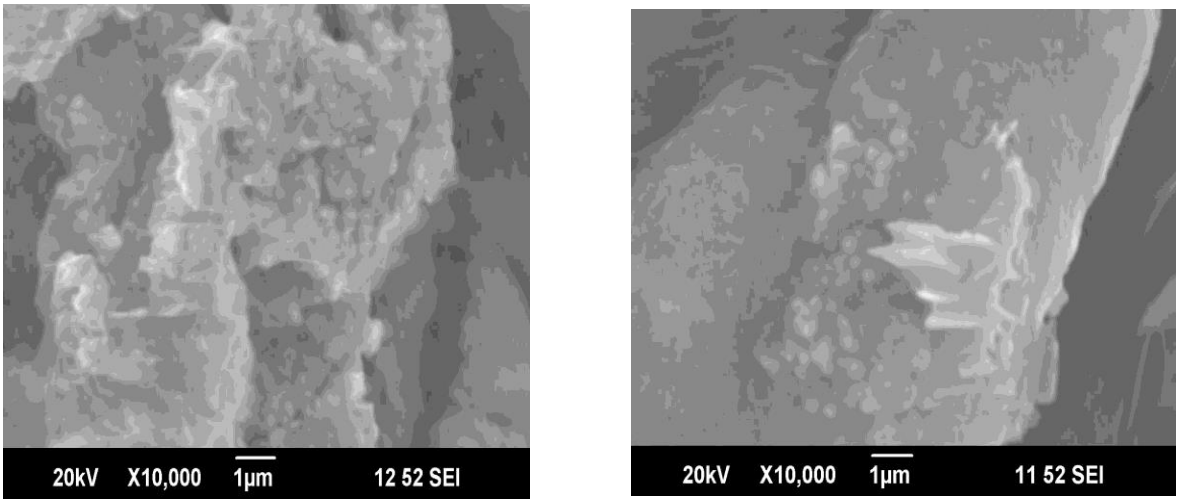


Fig- 3 a & b : TEM analysis of silver nanoparticle synthesized from fresh [a] and dried [b] rhizomes of Zofficinale

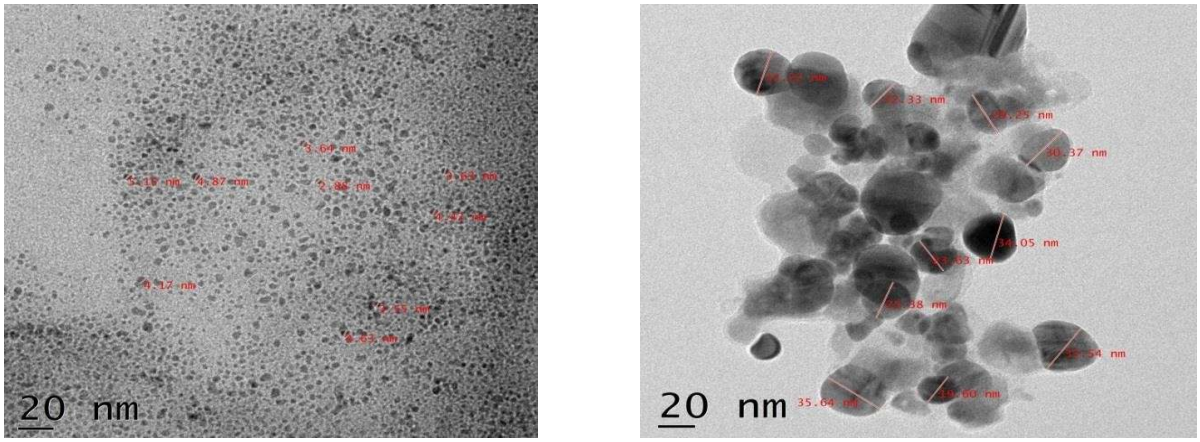


Fig- 4 a & b : TEM analysis of gold nanoparticle synthesized from fresh [a] and dried [b] rhizomes of Zofficinale

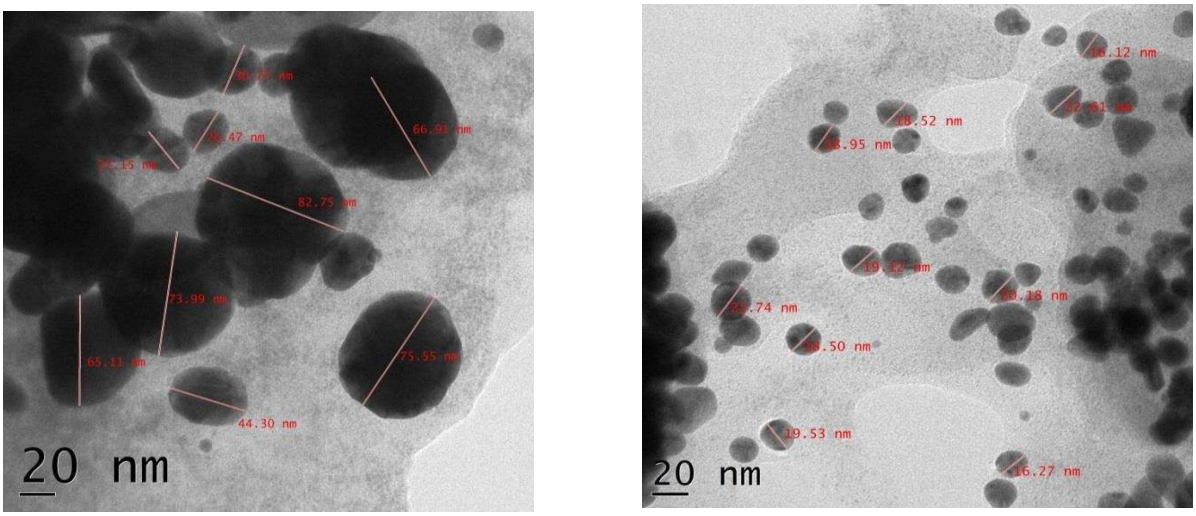


Fig 5:EPAX analysis of silver nanoparticle synthesized from fresh rhizomes of Z.officinale

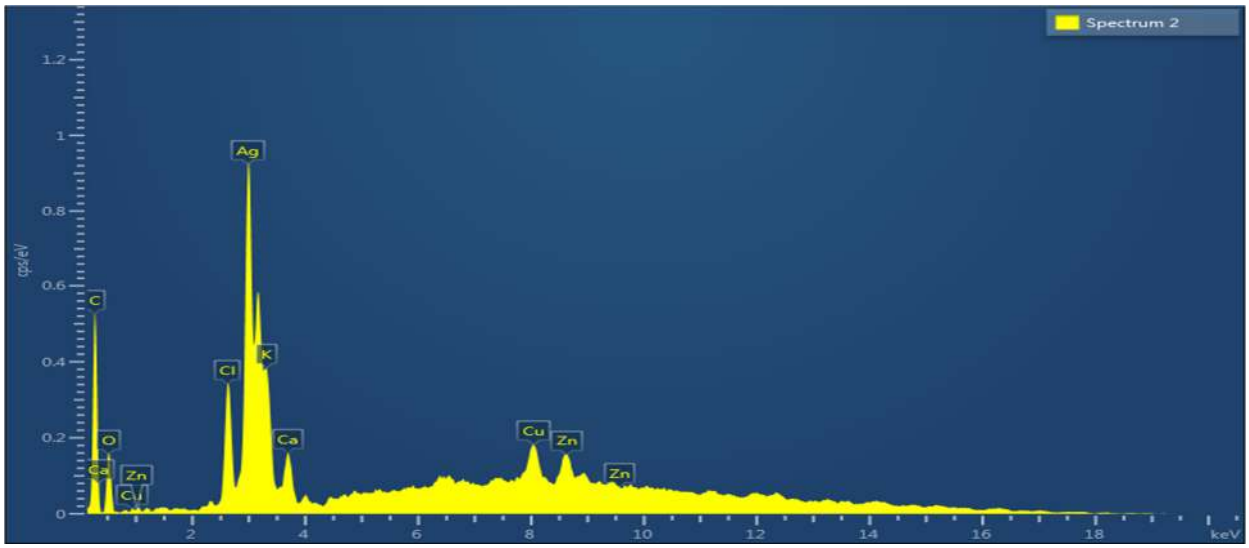


Fig 6 :EPAX analysis of silver nanoparticle synthesized from dried rhizomes of Z.officinale

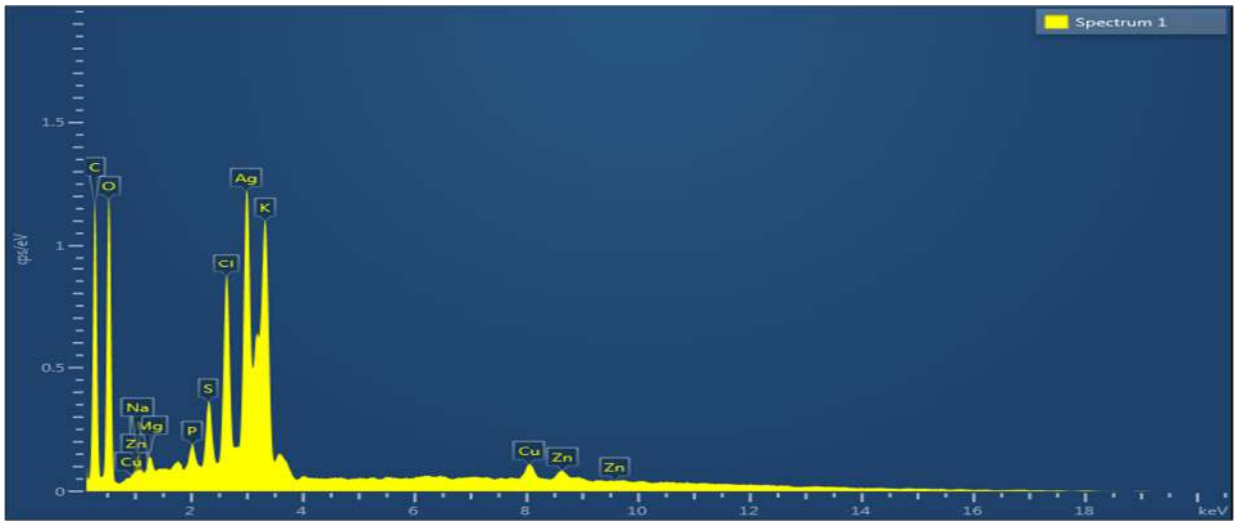


Fig7 : EPAX analysis of gold nanoparticle synthesized from fresh rhizomes of Z.officinale

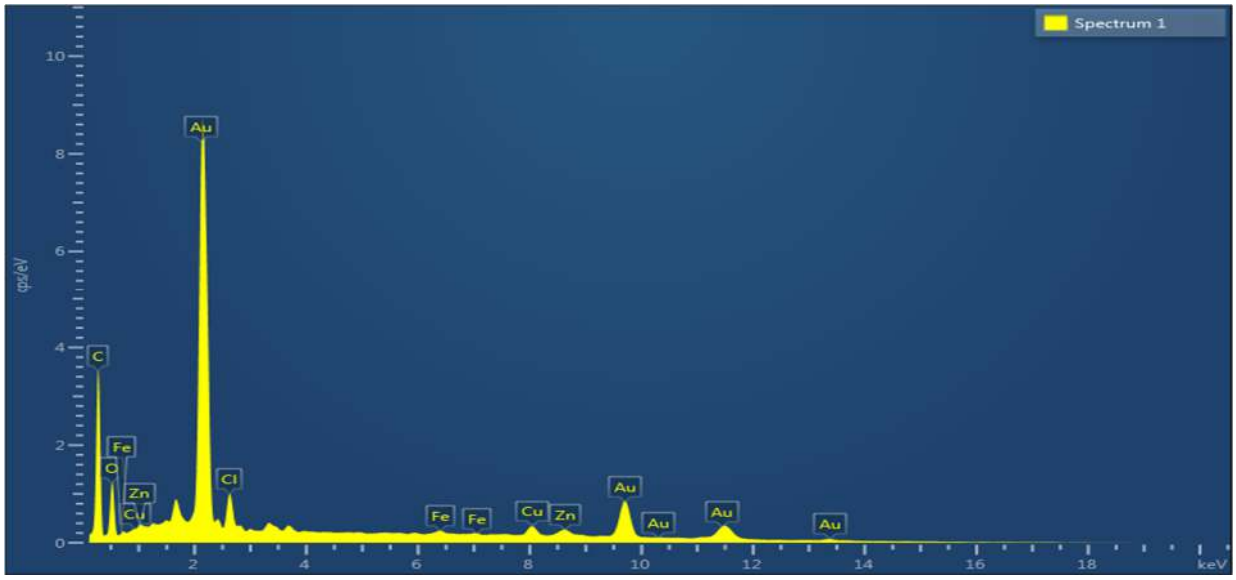


Fig 8 : EPAX analysis of gold nanoparticle synthesized from dried rhizomes of Z.officinale

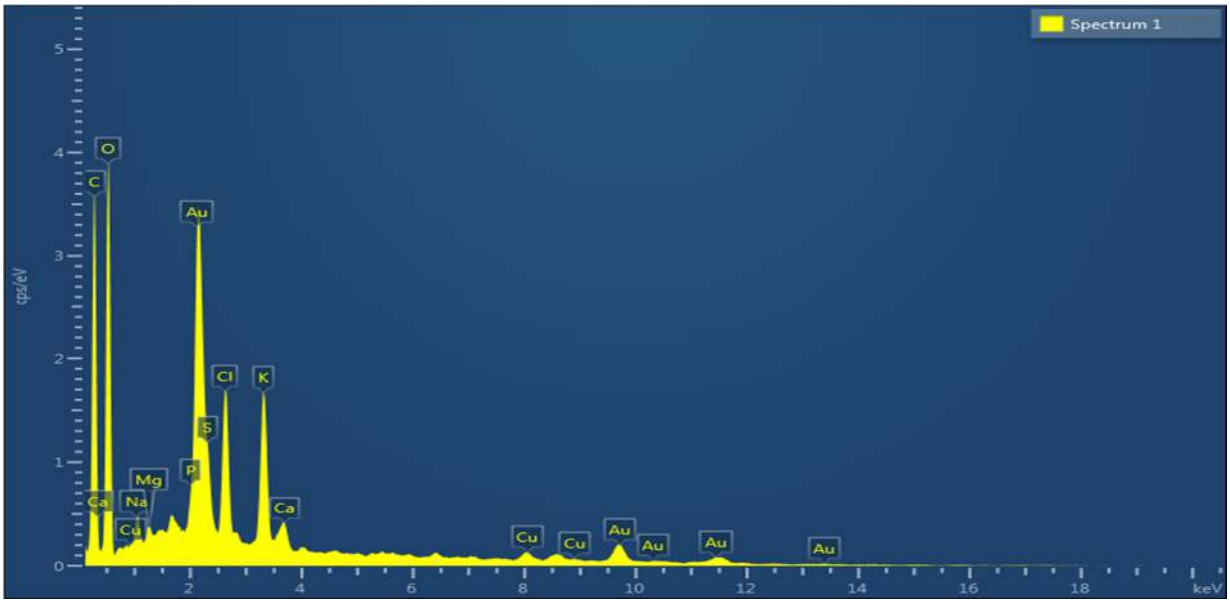


Table -1: % Inhibition (IC_{50} Value) of the Silver and gold nanoparticles of fresh and dried rhizomes of Z.officinale on PC-3; MCF-2, HepG2, HeLa and A 549 cell lines by MTT assay method.

Samples/Cell line	IC50 values µg/ml				
	PC-3	MCF-7	HepG2	HeLa	A549
GDGN	-	202.9	294.4	245.6	245.3
GDSN	77.78	79.05	78.58	104.3	97.31
GFGN	-	-	-	-	143.7
GFSN	117.4	131.1	102.2	97.83	74.26

-= IC50 was not calculated due to lesser inhibition

Fig 9: Graphical representation of IC_{50} value of the Silver nanoparticles of fresh and dried rhizomes of Z.officinale on PC 3 cell lines

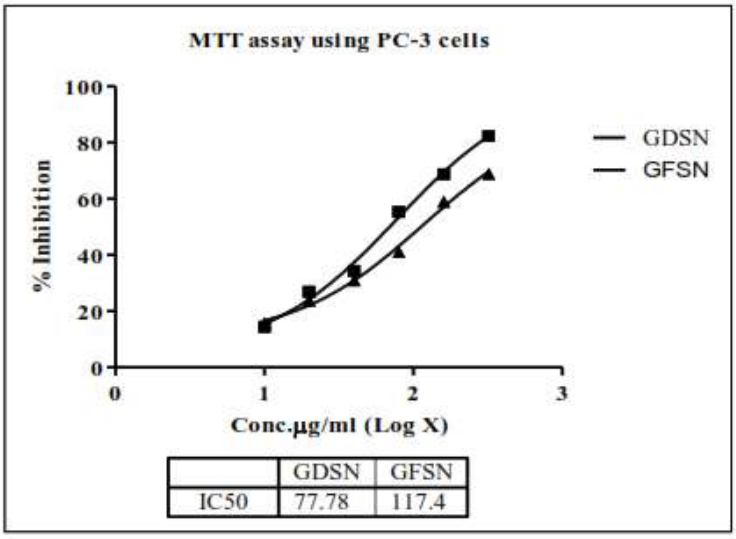


Fig 10: Graphical representation of IC_{50} value of the Silver and gold nanoparticles of fresh and dried rhizomes of Z.officinale on MCF-2 cell lines

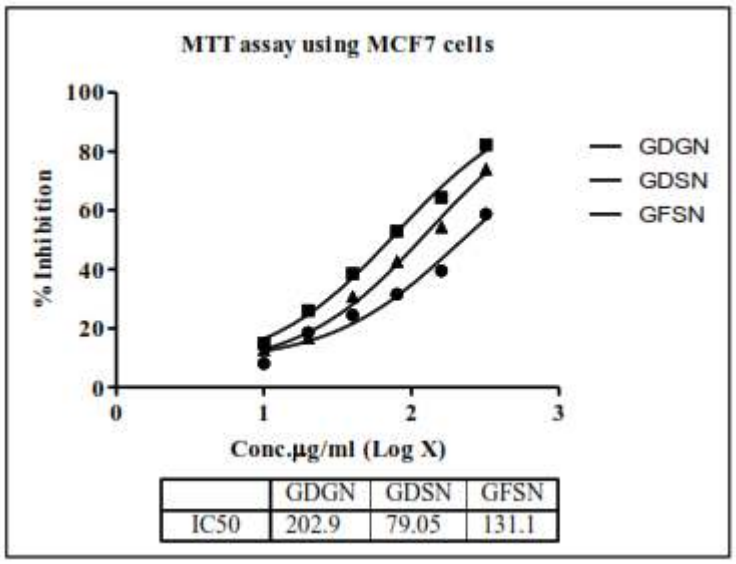
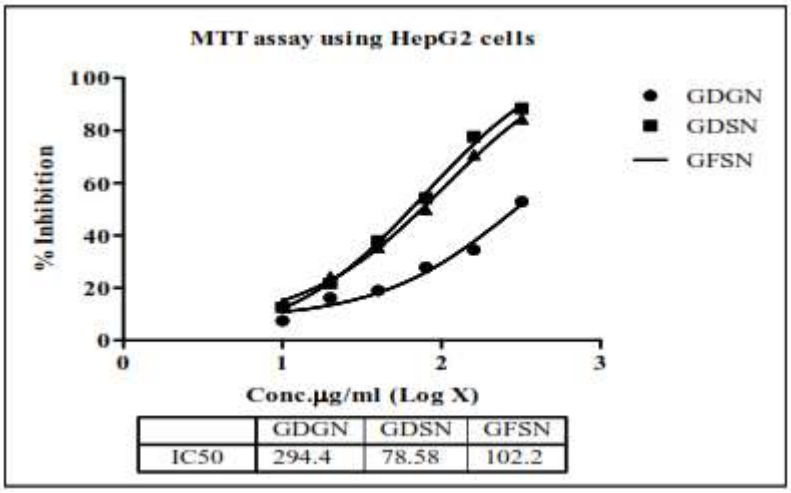
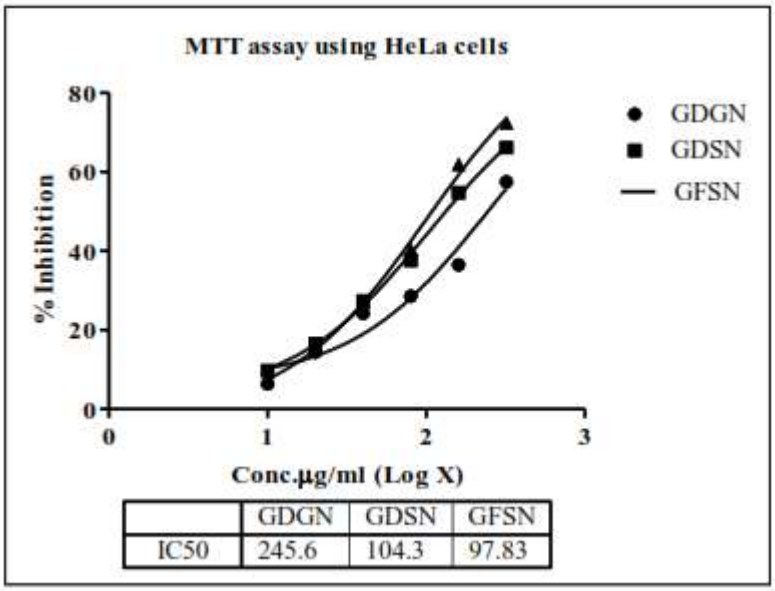


Fig 11: Graphical representation of IC_{50} value of the Silver and gold nanoparticles of fresh and dried rhizomes of Z.officinale on HepG2 cell lines



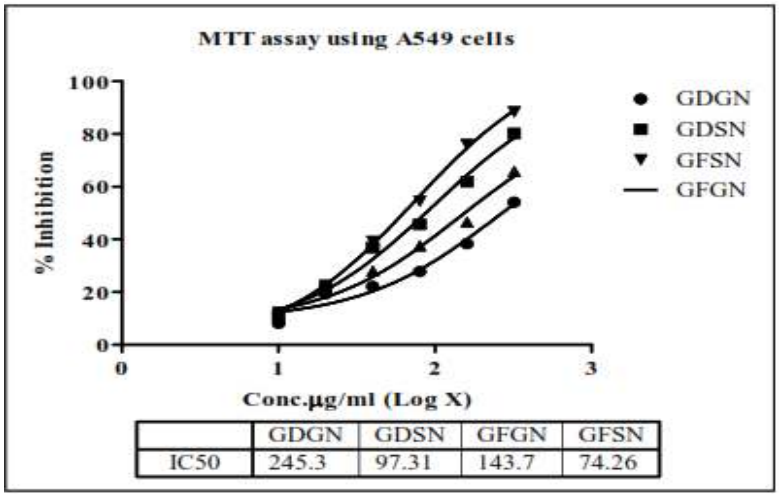
	GDGN	GDSN	GFSN
IC50	294.4	78.58	102.2

Fig 12: Graphical representation of IC_{50} value of the Silver and gold nanoparticles of fresh and dried rhizomes of Z.officinale on HeLa cell lines



	GDGN	GDSN	GFSN
IC50	245.6	104.3	97.83

Fig 13: Graphical representation of IC_{50} value of the Silver and gold nanoparticles of fresh and dried rhizomes of Z.officinale on A549 cell lines.



	GDGN	GDSN	GFGN	GFSN
IC50	245.3	97.31	143.7	74.26

Fig 14: Effect of GDSN,GDSN and GDGD on cell cycle of different cancer cell lines

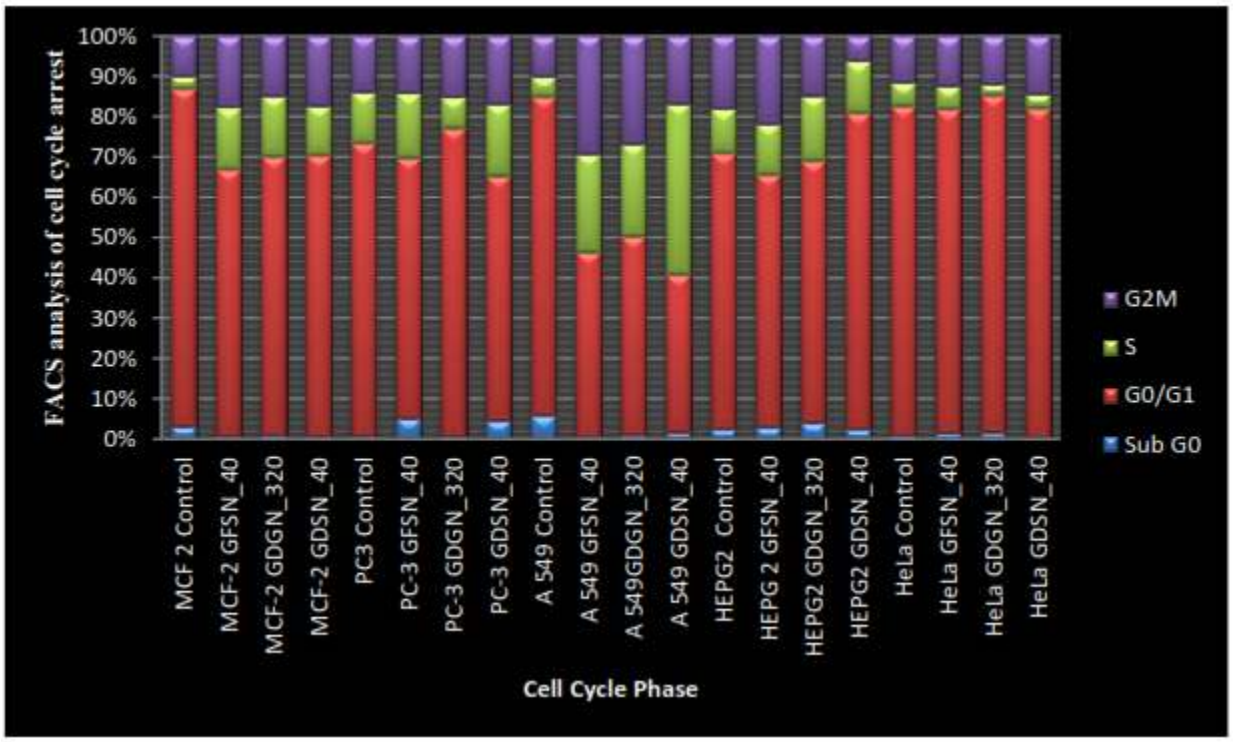
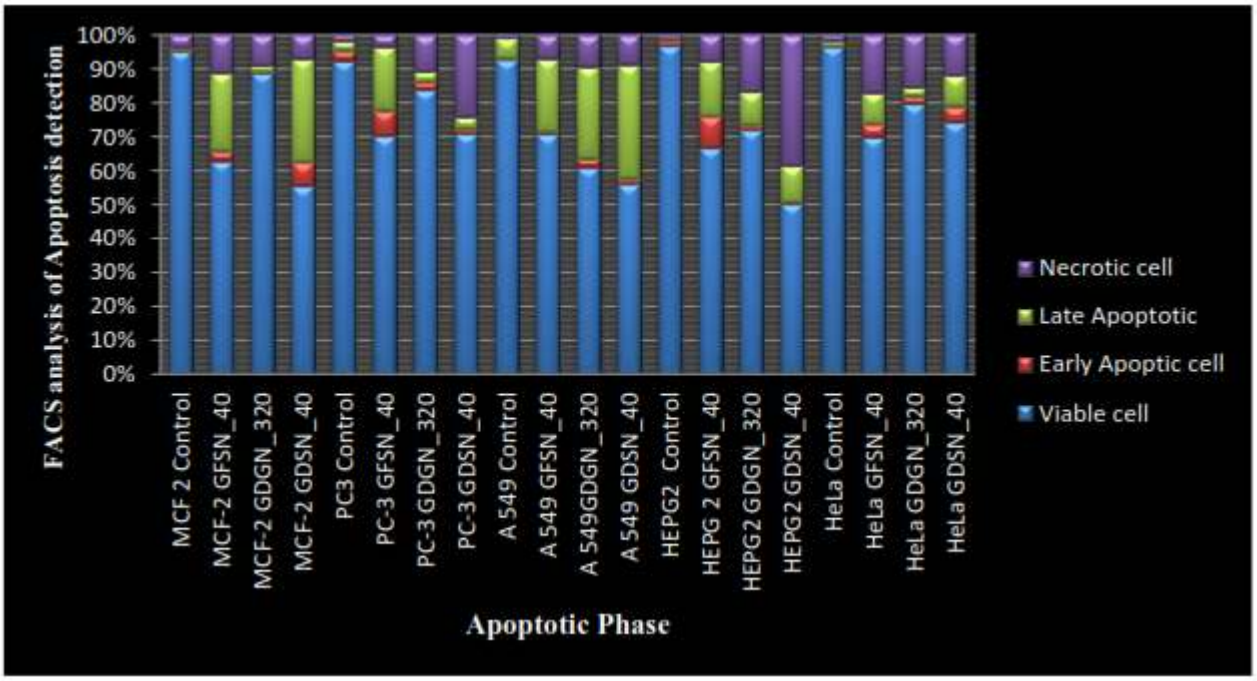


Fig 15: Effect of GDSN,GDSN and GDGD on apoptosis of different cancer cell lines



The cycle of increase in components (growth) and division, followed by growth and division of these daughter cells, etc., is called the cell cycle. The two most obvious features of the cell cycle are the synthesis and duplication of nuclear DNA before division, and the process of cellular division itself

-mitosis. These two components of the cell cycle are usually indicated in shorthand as the "S phase" and "mitosis" or "M".

When the S phase and M phase of the cell cycle were originally described, it was observed that there was a temporal delay or gap between mitosis and the onset of DNA synthesis, and another gap between the completion of DNA synthesis and the onset of mitosis. These gaps were termed G1 and G2, respectively. One of the earliest applications of flow cytometry was the measurement of DNA content in cells. This analysis is based on the ability to stain the cellular DNA in a stoichiometric manner. A variety of dyes are available to serve this function, all of which have high binding affinities for DNA. The location to which these dyes bind on the DNA molecule varies with the type of dye used. The most common DNA binding dye in use today is the blue-excited dye Propidium Iodide (PI). PI is an intercalating dye which binds to DNA and double stranded RNA (and is thus almost always used in conjunction with RNaseA to remove RNA). When diploid cells which have been stained with a dye that stoichiometrically binds to DNA are analysed by flow cytometry, a "narrow" distribution of fluorescent intensities is obtained. The study suggests that the cells treated with the samples have shown marked increase in % of cells in S phase & G2M phase compared to control; as the check points at these phases play a major role in DNA replication & cell division[10-13].

Table 2: Effect of silver and gold nanoparticles against cancers cell lines on cell cycle

	Viable cell	Early Apoptic cell	Late Apoptotic	Necrotic cell
MCF 2 Control	95.42	0.38	0.4	3.8
MCF-2 GFSN_40	62.96	2.78	23.12	11.14
MCF-2 GDGN_320	88.86	0.34	1.62	9.18
MCF-2 GDSN_40	55.58	6.94	30.48	7
PC3 Control	92.42	2.84	3	1.74
PC-3 GFSN_40	70.24	7.46	18.82	3.48
PC-3 GDGN_320	83.84	2.2	3.08	10.88
PC-3 GDSN_40	71	1.28	3.66	24.06
A 549 Control	92.76	0.19	6.09	0.96
A 549 GFSN_40	70.86	0.55	21.64	6.95
A 549GDGN_320	61.05	2.43	27.24	9.28
A 549 GDSN_40	56.12	1.52	33.65	8.71
HEPG2 Control	96.86	1.28	0.46	1.4
HEPG 2 GFSN_40	66.8	9.24	16.1	7.86
HEPG2 GDGN_320	72.1	1.28	9.8	16.82
HEPG2 GDSN_40	50.24	0.42	10.74	38.6
HeLa Control	96.6	0.14	1.68	1.58
HeLa GFSN_40	70.04	3.62	9.38	16.96
HeLa GDGN_320	79.76	2.04	2.7	15.5
HeLa GDSN_40	74.24	4.6	9.28	11.88

The cell cycle arrest on all cancerous cell line used treated with various samples is represented in the Fig 14 and Table 2. The study suggests that the cells treated with the samples have shown marked increase

in % of cells in S phase & G2M phase compared to control. The samples GDSN at 40 µg/ml has shown increased % of cells at S phase arrest up to 42.88% with 16.94% G2M arrest respectively compared to control A 549, 4.93% at S phase and 10.19% at G2M phase. The samples GDGN at 320µg/ml and GFSN at 40µg/ml have shown 23.14% and 24.64% arrest in S phase and 26.94% and 29.42% arrest in G2M phase. The study suggests that the samples tested on HeLA,MCF-2 and HEPG2 cells treated with the samples has shown no marked increase in % of cells in S phase or G2M phase compared to control. The sample GFSN and GDSN at 40 µg/ml has shown marked increase in cells in SubG0 arrest up to 5.22 and 4.64 % compared to control PC-3 cells, 0.68 % cells. The samples GDSN at 40 µg/ml have shown 17.90 % arrest in S phase and 16.92 % arrest in G2M phase. From the cell cycle analysis, it is clear that GFSN, GDSN and GDGN causes increase in S and G2M stage in A 549 and PC-3 suggesting them to have control on the cell replication and cell division.

Apoptosis is a cell death process characterized by morphological and biochemical features occurring at different stages. Once triggered apoptosis proceeds with different kinetics depending on cell types and culminates with cell disruption and formation of apoptotic bodies. A critical stage of apoptosis involves the acquisition of surface changes by dying cells that eventually results in the recognition and the uptake of these cells by phagocytes

Table 3: Effect of silver nanoparticles against cancers cell lines on Apoptosis

	Sub G0	G0/G1	S	G2M
MCF 2 Control	3.1	84.04	3.1	9.96
MCF-2 GFSN_40	0.64	66.78	15.5	17.76
MCF-2 GDGN_320	0.86	69.52	15.1	14.94
MCF-2 GDSN_40	0.55	70.2	12.34	17.32
PC3 Control	0.68	73.18	12.8	13.88
PC-3 GFSN_40	5.22	64.98	16.14	14.24
PC-3 GDGN_320	0.58	76.68	7.7	15.2
PC-3 GDSN_40	4.64	61.02	17.9	16.92
A 549 Control	5.86	79.63	4.93	10.19
A 549 GFSN_40	0.44	46.38	24.64	29.42
A 549GDGN_320	1.06	49.68	23.14	26.94
A 549 GDSN_40	1.82	39.52	42.88	16.94
HEPG2 Control	2.5	68.92	10.99	18.16
HEPG 2 GFSN_40	2.92	62.96	12.68	21.84
HEPG2 GDGN_320	4.12	65.35	16.22	14.89
HEPG2 GDSN_40	2.44	79.36	12.96	6.26
HeLa Control	0.82	82.14	5.8	11.56
HeLa GFSN_40	1.44	80.9	5.76	12.44
HeLa GDGN_320	1.76	83.74	2.82	11.98
HeLa GDSN_40	0.32	81.96	3.4	14.42

Different changes on the surface of apoptotic cells such as the expression of thrombospondin binding sites, loss of sialic acid residues and exposure of a phospholipid like phosphatidylserine (PS) were described.

Phospholipids are asymmetrically distributed between inner and outer leaflets of the plasma membrane with phosphatidylcholine and sphingomyelin exposed on the external leaflet of the lipid bilayer, and phosphatidylserine predominantly

observed on the inner surface facing the cytosol. Exposure of PS on the external surface of the cell membrane has been reported for activated platelets and senescent erythrocytes. Recently, it was shown that cells undergoing apoptosis break up the phospholipid asymmetry of their plasma membrane and expose PS which is translocated to the outer layer of the membrane. This occurs in the early phases of apoptotic cell death during which the cell membrane remains intact. This PS exposure may represent a hallmark (early and widespread) in detecting dying cells. Annexin V, belonging to a recently discovered family of proteins, the annexin, with anticoagulant properties has proven to be a useful tool in detecting apoptotic cells since it preferentially binds to negatively charged phospholipids like PS in the presence of Ca2+ and shows minimal binding to phosphatidylcholine and sphingomyeline.

Changes in PS asymmetry, which is analysed by measuring Annexin V binding to the cell membrane, were detected before morphological changes associated with apoptosis have occurred and before membrane integrity has been lost. By conjugating FITC to Annexin V it is possible to identify and quantitate apoptotic cells on a single cell basis by flow cytometry. Staining cells simultaneously with FITC-Annexin V (green fluorescence) and the propidium iodide (red fluorescence) allows the discrimination of intact cells, early apoptotic and late apoptotic or necrotic cells[13].

All cancerous cells were treated with various samples at various concentrations were analysed for apoptosis by Flow cytometer. The results are represented in the Table 3 and Fig 15. The samples GDSN at 40 µg/ml and GDGN at 320 µg/ml have shown 33.65 % and 27.24 % arrest in late apoptotic cell and 8.71 % and 9.28 % arrest in necrotic cells compared to control A 549 cells as 6.09 % and 0.96%. The samples GDSN at 40 µg/ml have shown 9.28 % arrest in late apoptotic cell and 11.88 % arrest in necrotic cells compared to control HeLa cells as 1.68 % and 1.58%. There is increase in necrotic cells in all treatment groups when compared to control. The samples GDSN at 40 µg/ml have shown 10.74 % arrest in late apoptotic cell and 38.60 % arrest in necrotic cells compared to control HEPG2 cells as 0.46 % and 1.40%. The samples GDSN at 40 µg/ml and GFSN at 40 µg/ml have shown 30.48% and 23.12 % arrest in late apoptotic cell and 7.00% and 11.14 % arrest in necrotic cells compared to control MCF-2 cells as 0.40 % and 3.80 %. The sample GFSN at 40 µg/ml have shown 18.22 % arrest in late apoptotic cell compared to control as 1.74 %. GFSN, GDSN and GDGN caused decrease in viable cancer cells, increase in the number of late apoptotic and necrotic cells suggesting to possess apoptotic activity. Ginger extract has potent anticancer activity against pancreatic cancer cells by inducing ROS-mediated autosis is reported not only for this cancer[14] and enriched use of Z. officinale as dietary material could be recommended in ethno-medicine for the management of cervical and breast cancers[15,16]. As suggested from the result of in vivo and in vitro and previous studies on *Z.officinale* has shown that silver and gold nanoparticle has very good activity against cell lines. It is well known that high amount of ROS generation could lead to apoptotic and necrotic cell death.[17] Excessive ROS generation has been linked with the substantial DNA damage and apoptosis/necrosis.[18] Our results are in well accordance with the recent reports that have shown apoptosis cell death due to the exposure of nanoparticles including the exposure of plant-synthesized silver nanoparticles.

CONCLUSION

The cytotoxicity study, inhibition of cell division and increase in number of late apoptotic cell and necrotic cell clearly suggest the role of silver and gold nanoparticle in prevention of proliferation of cancer cells. In future we have planned to target the silver and gold nanoparticle at the site of cancer cell itself. Thus, silver and gold nanoparticles synthesised using the fresh and dried rhizomes of *Z.officinale* is promising agent for Nano chemoprevention of various cancer cell used for the study.

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REFERENCES

1. Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. Acc Chem Res, 2008; 41(12):1578–1586.
2. Afsar T, Trembley JH, Salomon CE, Razak S, Khan MR, Ahmed K. Growth inhibition and apoptosis in cancer cells induced by polyphenolic compounds of *Acacia hydaspica*: Involvement of multiple signal transduction pathways. Sci Rep, 2016; **6**: 23077.
3. Herbst RS, Heymach JV, Lippman SM .Lung cancer. N Engl J Med 2008; 359:1367– 1380.
4. Shaikh I, Khaleequr R, Arshiya S, Mohd T, Shahid S. *Zingiber officinale* Rosc.: A traditional herb with medicinal properties.Tang Humanitas Trad Med, 2013;3(4):e26
5. Anonymous. Quality Standards of Indian Medicinal Plants, Vol 8, Indian Council for Medical Research, New Delhi, 2003,pp.347-356.
6. Padmaa MP. Green synthesis of silver nanoparticles using fruits of *Coriandrum sativum* Linn and its antioxidant activity. J Nat Prod Res, 2015; 1(1): 19-22.
7. Mosmann TT. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immun Meth, 1983; 12-16.
8. Joany J, Patrick MC. Methods for Synchronizing Cells at Specific Stages of the Cell Cycle. Current Protocols in Cell Biology, 1998; 8.3.1-8.3.20.
9. VanEngeland M, Ramaekers FC, Schutte B, Reutelingsperger CPM. A novel assay to measureloss of plasma membrane asymmetry during apoptosis of adherent cells in culture. Cytometry, 1996; 24:131-139. Vajihe AZ, Ghamartaj H, Ehsan J. Effect of lithium chloride and anti neoplastic drugs on survival and cell cycle of androgen-dependent prostate cancer LNCap cells. Ind J Pharmacol, 2012; 44(6): 714-721.
10. Ramesh P. Thylur, Senthivinayagam S, Campbell EM, Rangasamy V, Thorenoor N, Sondarva G, Mehrotra S, Mishra P, Zook E, Le PT, Rana A, Rana B. Mixed Lineage Kinase 3 Modulates β-Catenin Signaling in Cancer Cells. J Biol Chem, 2011; 286(43):37470-82.
11. Kasibhatla S, Amarante-Mendes G, Finucane S, Brunner T, Bossy-Wetzel E, Green D R. Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. Cold Spring Harbor Protocols, 2006*;* (3), pdb-prot449
12. Foldbjerg R, Olesen P, Hougaard M, Dang DA, Hoffmann HJ, Autrup H, PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis, and necrosis in THP-1 monocytes. Toxi Lett, 2009; 190(2):156–162.
13. Rahman S, Salehin F, Iqbal A. *In vitro* antioxidant and anticancer activity of young *Zingiber officinale* against human breast carcinoma cell lines. BMC Complement Altern Med, 2011; 11:76.
14. Akimoto M, Iizuka M, Kanematsu R, Yoshida M, Takenaga K. Anticancer Effect of Ginger Extract against Pancreatic Cancer Cells Mainly through Reactive Oxygen Species-Mediated Autotic Cell Death. PLoS ONE,2015; 10(5): e0126605.
15. Ansari, J,Ahmad, M Khan, A,Fatima, N, Khan, H, Rastogi N, Mishra D, Mahdi AA.Anticancer and Antioxidant activity of *Zingiber officinale* Roscoe rhizome. Ind J Exp Bio, 2016; 54:767-773
16. Pan Y, Neuss S, Leifert A.Size-dependent cytotoxicity of gold nanoparticles. Small 2007; 3(11): 1941–1949.
17. Stroh A, Zimmer C, Gutzeit C.Iron oxide particles for molecular magnetic resonance imaging cause transient oxidative stress in rat macrophages. Free Rad Bio Med, 2004; 36(8):976–984.

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