CHAPTER 9

ANTICANCER ACTIVITY OF GEDUNIN FROM AZADIRACHTA INDICA AGAINST NON-CANCEROUS CELL LINE, LIVER CANCER CELL LINE HEPG2, PA1 OVARIAN AND PC3 PROSTATE CANCER CELLS

9.1 Introduction

Ovarian cancer accounts for approximately 4 percent of female cancer cases and 528 new cases per 100,000 women yearly record breast neoplasms. Since ancient times, herbal treatments have demonstrated broad benefits and great potential for protecting human health. *Azadirachta indica* (neem) is an Indian medicinal plant with more than 140 isolated compounds and a minimum of 35 biologically active ingredients that, by interfering with the carcinogenesis process, showed a significant effect as tumour suppressors. Neem chemicals in bark, leaves, flowers, and seed oil have been shown to possess characteristics such as chemoprevention, apoptosis, immunomodulation, and p53-independent apoptosis induction, which have been a natural treatment for cancer in Asia for many generations (Moga **et al., 2018).** The few bioactive components of neem that have been widely explored are azadirachtin and nimbolide. However, it is necessary to investigate many more bioactive components. The primary anticancer actions of neem on malignant cells include cell growth, cell death induction and abolition, cell reduction/oxidation (redox), and enhancement of the host's immunological response to tumour cells. The most significant anticancer effect is the inhibition of cell proliferation. Although the underlying processes are primarily unknown, removing the NF-κB

signalling pathway has partially been implicated in neem-component anticancer function. Essentially, the actions of neem components, which are anti-proliferative and apoptosis-inducing, are tumor-selective since they dramatically reduce the effect on normal cells. Neem extracts also increased the sensitivity and effectiveness of several chemical immunotherapy agents and radiation therapy in cancer cells**.** Gedunin is a natural secondary tetranorterpenoid discovered in the Meliaceae family of plants owing to its antiparasite, antifungal, and anticancer properties. Gedunin showed anti-proliferative action in cell lines, including NTERA-2 prostate and SKOV3, OVCAR4, and OVCAR8 ovarian cancer cell lines.

Hepatic carcinoma is the most frequent fatal disease worldwide. Patients typically have an advanced liver cancer diagnosis, contributing to a poor prognosis. There have been far from successful therapeutic methods, such as surgical resection and liver transplantation. Therefore, new therapeutic techniques are needed. This study discussed the process of development and treatment objectives for liver cancer. Currently, the therapy is confined only to treatment with sorafenib, lenvatinib, regorafenib, ramucirumab, and cabozantinib, which, however, is impaired by the presence of drug resistance in patients and contributes to survival benefits **(Man *et al.,* 2021).**

Here anticancer activity of gedunin from *azadirachta indica* against liver cancer cell line HepG2 covers the anticancer property of gedunin, which has been explored using liver cancer cell by performing MTT assay, oxidative stress assay, and apoptosis assay through flow cytometry.

Despite having a lower frequency than breast cancer, ovarian cancer is three times more lethal **(Lan *et al.,* 2013)**, and the death rate from this illness is expected to

increase dramatically by 2040 **(Zheng *et al.*, 2011).** Ovarian cancer has a high death rate because of silent and hidden tumor development, delayed onset of symptoms, and a lack of effective screening, which results in detection in advanced stages **(Cragg *et al*., 1997).** As a result, this malignancy has been given the moniker "silent killer."

Like many other malignancies, ovarian cancer has a global occurrence **(Kharhadakar *et al.*, 2004).** The risk factors contributing to ovarian cancer incidence are responsible for the epidemiological variety of ovarian cancers in different places **(Batsaikhan *et al.*, 2014).** Ovarian cancer is most common in non-Hispanic white women (12.0 per 100,000), followed by Hispanic women (10.3 per 100,000), non-Hispanic black women (9.4 per 100,000), and Asian/Pacific Islander women (9.4 per 100,000), (9.2 per 100,000) **(Chung *et al.*, 2015).** Ovarian cancer mortality follows a varied pattern due to disparities in access to diagnostic and treatment services, with African people having the greatest fatality rate. According to statistics, one-third to two-fifths of all cancer cases can be avoided by removing or decreasing the risk factors. Knowledge of the incidence, mortality, and geographic diversity of ovarian cancer, as well as its risk factors, is essential for planning and preventing complications.

Prostate cancer is a complicated illness that affects millions of men worldwide, mostly in areas with a high human development index. Suppose the cancer is discovered and treated early enough. In that case, patients with localized disease at a low to moderate risk of recurrence have a 99% OS rate for ten years. The fusion of TMPRSS2 with ETS family genes, amplification of the MYC oncogene, deletion or mutation of PTEN and TP53 and in late illness, amplification and mutation of the androgen receptor (AR) are among the most common genetic

changes. Blood tests to evaluate prostate-specific antigen levels and digital rectal examinations are commonly used to identify prostate cancer.

Active surveillance, radical prostatectomy, and ablative radiation are all options for treating localized illnesses. For local relapse, men are treated with salvage radiation and androgen deprivation treatment (ADT), whereas systemic relapse is treated with ADT combined with chemotherapy or new androgen signaling– targeted drugs. Prostate cancer that has progressed despite androgen ablation is castration-resistant and incurable. AR-targeted medicines, chemotherapy, radionuclides, and the poly(ADP-ribose) inhibitor olaparib are currently available therapeutic options. Current research aims to identify the marker protein which may help in prostate cancer diagnosis **(Shen *et al.,* 2005)**.

Cancer stem cells (CSCs) are the starting point for tumor growth and progression **(Kakarala *et al.,* 2010)**. Stem cell properties include the proliferation, self-renewal, and differentiation of the original tumor into all cell types **(Patwardhan *et al.,* 2013)**. CSCs resist traditional chemotherapy, radiation therapy, and various natural and synthetic anticancer medicines, resulting in cancer recurrence following standard therapies **(Uddin *et al.,* 2007)**. As a result, it is critical to find new treatment strategies that target cancer-dependent malignant cells and CSCs without harming healthy cells **(Kamath *et al.,* 2009)**. Because CSCs have distinct dynamics and characteristics, they can be targeted using various techniques, including sensitization to natural or synthetic chemicals, induction of differentiation into other cell types, and self-renewal restriction **(Santagata *et al.,* 2012)**.

Natural compounds originating from plants and microbes account for over 60% of clinically approved anticancer medicines **(Mayan *et al.,* 2016)**. Signaling

pathways regulated by Hedgehog, Wnt/-catenin, and Notch are thought to be critical for CSC differentiation and self-renewal **(Azouaou *et al.,* 2015)**. Several natural substances target CSC signaling pathways. Hedgehog signaling is targeted by cyclopamine, an alkaloid isolated initially from Veratrum californicum **(Samarakoun *et al.,* 2014)**. One of the key components in tea, epigallocatechin gallate (EGCG), has been shown to block the Wnt/-catenin signaling pathway **(Samarakoun *et al.,* 2012)**. Vitamin D and its analogs have also been shown to suppress the Notch and Wnt/-catenin signaling pathways **(Ediriweera *et al.,* 2016)**. Curcumin and piperine, well-known anticancer drugs, have been shown to target breast CSCs **(Kakarala *et al.,* 2010)**.

One of the critical chemical substances identified in the neem tree is gedunin (a tetranortriterpenoid) **(Boe *et al.,* 2016)**. Gedunin has been shown to limit the growth of cancer cells in the NTERA-2 prostate and SKOV3, OVCAR4, and OVCAR8 ovarian cancer cell lines in recent investigations **(Livak and Yiang, 2014)**. Hsp90 of heat shock protein inhibition has also been found **(Jiang *et al.*, 2014).** Furthermore, the pharmacological similarity of gedunin to -catenin chain A in cancer stem cells was discovered in a recent *in silico* study **(Li *et al.,* 2008).** However, no systemic study has been made on prostate and ovarian cancers using gedunin as an anti-cancer agent.

9.2 Experimental

9.2.1 Molecular Docking

Gedunin with PubChem ID: 12004512 was docked onto the target alpha-fetoprotein (AFP) marker, PDB id 6M4R for HepG2 cells and CEA marker, PDB id 2QSQ for PA1 AND PC3 cell line. The structures of the enzymes and ligands

were obtained from the Protein Data Bank, RCSB database, and National Centre for Biotechnology Information, PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Molecular docking is an *in silico* study of the intermolecular interactions between two molecules **(Saikia *et al.*, 2019).** A ligand molecule is a small molecule that can serve as an inhibitor. Ligand was downloaded from PubChem.

The three-dimensional structure of the protein was obtained from the Protein Data Bank (PDB), which allowed the removal of water molecules from the cavity, the stabilisation of charges, the filling of missing residues, and the formation of side chains. Water molecules and heteroatoms were removed if they were present. The protein is docked with the ligand, and the interactions were investigated **(Lohning *et al* ., 2017).**

The docking procedure in the autodock was as follows:

Step 1: Protein preparation: The protein's three-dimensional structure was downloaded from the Protein Data Bank (PDB), and then the structure was pre-processed. According to default parameters, water molecules were removed from the cavity, stabilizing charges, filling missing residues, and creating side chains.

Step 2: Predicting the protein's active site: The protein's active site was predicted after protein preparation. Water molecules and heteroatoms present were eliminated. **(Kamath *et al.*, 2009)**.

Step 3: Ligand preparation: Ligands were downloaded from PubChem. The lipinski rule of 5 was evaluated with the ligand for drug-likeliness properties. The Lipinski rule of five can help distinguish between non-drug-like and drug-like candidates. This guarantees a high possibility of success or failure owing to the

pharmacological similarity of compounds following two or more rules **(Lohning *et al* ., 2017)**.

Step 4: Docking: The ligand is docked to the protein, and the interactions are examined. The scoring function assigns a score based on which docked ligand complex was selected as the best.

9.2.2 Simulation

Molecular dynamics is a method that employs classical mechanics to examine the structure of a compound/solid and produce a trajectory. MD was created using Gromacs, which uses the notion of periodic boundaries to create boxes/grids and groups to depict motion. After arranging several data files using the OPLS force field (conf. gro for organizes, Topol. top for topology, and the raw parameter file, grompp. mdp), the first step in employing GROMACS was to execute the preprocessing step, followed by the principal energy minimization software mdrun. Reproduction may be accomplished using selected checkpoint files and ensuring minimum procedures **(Hess *et al* ., 2005)**.

9.2.3 MTT Assay

Material

1. HepG2 Human hepatocellular carcinoma cell line, PA1- Human ovarian cancer cell line and PC3- Human prostate cancer cell line were collected from NCCS Pune, India.
2. Cell culture media – Minimum essential medium with Earle‟s Salts And Non-Essential Amino acids supplemented with 10% Foetal Bovine serum (FBS), MP Biomedicals, Germany

3. 1X Dulbecco‟s phosphate-buffered saline (DPBS), 0.25% trypsin-EDTA solution and MTT reagent were all purchased from MP Biomedicals, Germany
4. Dimethyl Sulfoxide (DMSO), research grade, Merck, Germany
5. Cell culture treated T-25 flasks from Biolite, Thermo Fisher Scientific Inc., USA.
6. 10mL serological pipettes and 96-well plates from Nunc, Thermo Fisher Scientific Inc., USA.
7. 5mL, 2mL, and 1.5mL tubes, Tarsons, India.
8. Microplate reader - EL10A, Biobase, China
9. Microscope - XDFL series, Sunny Instruments, China

Method

Cells cultured in T-25 flasks were trypsinized and aspirated into a 5 mL centrifuge tube. Cell pellets were obtained by centrifugation at 300 g. The cell count was adjusted using a culture medium such that 200µl of suspension contained approximately 10,000 cells. To each well of the 96-well microtitre plate, 200µl of the cell suspension was added, and the plate was incubated at 37°C in a 5% CO_2 atmosphere for 24 h. After 24 h, the spent medium was aspirated. 200µl of Different test concentrations of test drugs were then added to the respective wells. The plate was then incubated at 37°C in a 5% CO_2 atmosphere for **24 hours**. The plate was removed from the incubator, and the drug-containing media was aspirated. 200µl of medium containing 10% MTT reagent was added to each well to obtain a final concentration of 0.5mg/mL. The plate was incubated at 37°C in a 5% CO_2 atmosphere for three hours **(Uchil *et al* ., 2018).** The culture medium was eradicated without disturbing the crystals formed. Then 100µl of solubilization

solution (DMSO) was added. The plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at 570 nm and 630 nm. The percentage growth inhibition was calculated after subtracting the background and the blank, and the concentration of the test drug needed to inhibit cell growth by 50% (IC_{50}) was generated from the dose-response curve for the cell line **(Buranaamnuay *et al.*, 2021).**

9.2.4 ROS Activity Detection

Material

1. HepG2 Human hepatocellular carcinoma cell line, PA1- Human ovarian cancer cell line and PC3- Human prostate cancer cell line were collected from NCCS Pune, India.
2. 2',7'-dichlorofluorescein diacetate (H_2DCFDA) (4 mM stock in DMSO) (Life Technologies, Invitrogen^{TM}, catalogue number: D-399, Thermo Fisher, USA)
3. Dimethyl Sulfoxide and $H2O2$, HiMedia, India
4. Dulbecco's Phosphate-buffered saline (DPBS), HiMedia, India
5. 5 ml RIA round-bottom tube with cell strainer cap, Tarsons, India
6. 5ml sterile centrifuge tubes - Tarsons, India
7. 1x Dulbecco's phosphate-buffered saline (DPBS) - HiMedia, India
8. Trypsin-EDTA solution–HiMedia, India
9. Cytomics FC500 Flow cytometer, Beckman Coulter, USA
10. Analysis software – FlowJo X 10.0.7

METHOD

Cultures of 3 x 10 5 cells/2 ml) were grown in 6-well plates and incubated in a CO_2 incubator at 37°C for 24 hours. The medium was washed and 1 ml of 1X PBS was added. Cells were treated in a culture medium (2 mL with appropriate concentrations of experimental (non-toxic) chemicals) and incubated for 24 h **(Venditti *et al*., 2019).** One of the wells was used as a negative (untreated) control. Wash with 1 ml of PBS and aspirate the medium. Challenge the cells in 2 mL of culture medium in 100 M $_{H2O2}$ and induce ROS stress for 2 h. One of the wells was used as a negative (untreated) control. After treatment, cells were transferred to 5 ml FACS tubes and washed with 1 ml PBS by trypsinization. Centrifuged at 300 x g for 5 minutes at 25 °C and washed twice with PBS. PBS was fully inclined. Diluted the H_2DCFDA solution (4 mM) in DPBS to obtain a 10 M working solution. Cell suspension in 100 µl of H_2DCFDA working solution at a density of 1 x 106 cells/ml and ink protected from light at 37° C for 30 minutes **(Shadel *et al*., 2015)** 150 x g for 5 minutes. Centrifuged the tube, removed the cells and resuspended in 400 µL of pre-warmed DPBS. The assay was analyzed by flow cytometry using a 488 nm laser for excitation and detection at 525 nm (FL1).

9.2.5 Apoptosis Assay

Material

1. HepG2 Human hepatocellular carcinoma cell line, PA1- Human ovarian cancer cell line and PC3- Human prostate cancer cell line were collected from NCCS Pune, India.
2. 5ml sterile centrifuge tubes - Tarsons, India

3. The cells were cultured in 6-well plates and T-25 flasks from Biolite (Thermo Fisher Scientific Inc., USA).
4. 1x phosphate-buffered saline (PBS), MP Biomedicals, Germany
5. Trypsin-EDTA solution – HiMedia
6. 2% Paraformaldehyde solution – HiMedia
7. 0.5% BSA in 1X PBS - HiMedia
8. 0.1% Triton-X 100 in 0.5% BSA solution - HiMedia
9. FITC Rabbit Anti-Active Caspase-3 IgG Antibody - BD Biosciences, Catalog No. 559341
10. Cytomics FC500 Flow cytometer, Beckman Coulter, USA
11. Analysis software – FlowJo X 10.0.7

METHOD

A culture of 3 × 105 cells/2 ml was cultured in a 6-well plate and incubated at 37° C overnight in a CO_2 incubator for 24h. The medium was washed, and 1 ml 1X PBS was added. The cells were treated in 2 ml of culture media with the necessary concentration of test compounds and incubated for 24 h **(Fan *et al*., 2017).** One of the wells was used as a negative control (untreated). After treatment, the medium was removed from each source and washed in 5 ml centrifugal tubes containing 500 µl PBS. The PBS and 200 µl of EDTA-trypsin, 37°C for three-four minutes, were Incubated. The restoration was done of the culture media in the centrifuge tubes in the corresponding wells and harvesting cells. Centrifugation time was five minutes at 25°C at 300 x g. decanted the supernatant and Washed 1x PBS twice. PBS decanted completely. The paraformaldehyde solution was added to 0.5 mL of 2 and allowed to simmer for 20 min. The cells were washed

with 0.5% bovine (BSA) in (PBS). In 0,1% Triton, the Triton-X100 solution and ink in 0,5% of the BSA solution for 10 min. Washed twice with 0,5% of (BSA) in (PBS). Added (PBS) and 20µl (BSA) anti-caspase-3 antibodies at 0.5%. Combined thoroughly and incubate in the dark for 30 min at room temperature (25 °C). PBS 0.5 ml was added, mixed and thoroughly examined. Shortly before the analytical mixing, the samples were not inspected immediately **(Peng *et al.,* 1997).**

9.2.6 Scratch assay / Cell migration assay.

Material

1. Cell line – **PC3 Human prostate cancer cell line, HepG2 Human liver cancer cell line and PA-1 Human ovarian cancer cell line and NIH/3T3 non-cancerous cell line, NCCS Pune**
2. Cell culture media – Dulbecco's Modified Eagle Medium with High Glucose (DMEM-HG) supplemented with 10% Foetal Bovine Serum (FBS).
3. T-75 Cell culture flasks – Biolite, Thermo Scientific
4. 6-well cell culture plates - Nunc, Thermo Scientific
5. Phosphate-Buffered saline (DPBS) - HiMedia
6. Trypsin - HiMedia
7. Sterile 5ml/15 ml centrifuge tubes – Tarsons
8. Microscope - XDFL series, Sunny Instruments, China
9. Analysis Software – ImageJ (Fiji) software, MRI Wound Healing Tool plugin.

METHOD

Culture cells in 6-well plates to form a monolayer. As the cells reach around 7080% confluence, they make a scratch to form a wound. Wash the monolayer with 1 ml DPBS two times. Add 2ml medium to each well with or without test drugs and incubate for 24h or 48h, depending on the cell migration rate. Take images at regular time intervals (0h, 12h, 24h & 48h as applicable) using an inverted phase contrast microscope. The percentage of cell migration is calculated by comparing the final gap (24h or 48h) area to the initial gap area (0h).

9.2.7. Colony formation assay/cell proliferation assay.

Materials

1. Cell line – **PC3 Human prostate cancer cell line, HepG2 Human liver cancer cell line and PA-1 Human ovarian cancer cell line, NCCS Pune**
2. T-75 Cell culture flasks – Biolite, Thermo Scientific
3. 6-well cell culture plates - Nunc, Thermo Scientific
4. Phosphate-Buffered saline (DPBS) - HiMedia
5. Trypsin - HiMedia
6. Sterile 5ml/15 ml centrifuge tubes – Tarsons
7. Microscope - XDFL series, Sunny Instruments, China
8. Analysis Software – ImageJ (Fiji) software

METHOD

The clonogenic assay was performed as described earlier with minor modifications for determining the anti-neoplastic effects against cell proliferation in vitro. In brief, the cells were cultured in T-25 tissue culture flasks for 24 hours and exposed to test compounds for 24h. The cells were then trypsinized, counted

and seeded into a 6-well plate (500-1000 cells/2mL medium per well) and cultured for 8 days. Colonies were fixed with methanol, stained with crystal violet (0.4 g/L), photographed, analyzed and counted using ImageJ (v1.48) software. The surviving cell fraction was calculated using the formula:

$$\text{Plating efficiency (PE)} = \frac{\text{No. of cells plated}}{\text{No. of colonies counted}} \times 100$$

$$\text{Surviving fraction (SF)} = \frac{\text{PE of treated sample}}{\text{PE of control sample}}$$

9.3 Result and Discussion

9.3.1 Molecular Docking

The best binding pose was selected by considering the lower binding energies among the poses. Images of the best pose were captured with the lowest energy score of -9.38. Docking resulted in the interaction of alpha-fetoprotein and gedunin at the active site with hydrogen bonds, namely TYR A:151, GLN A:63, ASP A:300and pi-pi stacked bond as well as alkyl bond, namely HIS:305, TRP A:59 and LEU A:165 as shown in **Figure 9.1**. Images of the best pose were captured with the lowest energy score of -7.96. Docking resulted in the interaction of CEA (2QSQ) and gedunin at the allosteric site with alkyl and hydrogen bonds, namely ILE A:50, LEU A:28, and HIS A:27, as shown in **Figure 9.2.** The active sites of 2QSQ include PHE 29, ILE 91, and LEU 95, as shown in **Figure 9.3**.

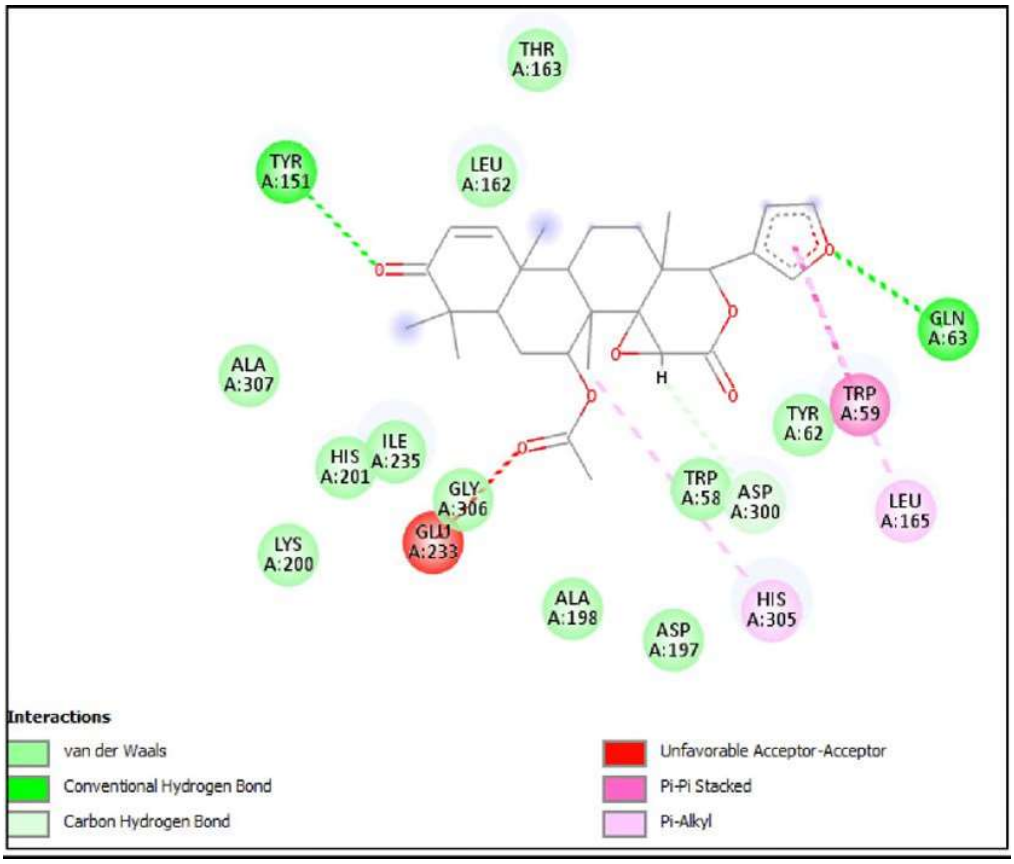


Figure 9.1. Ligplot of the docked structure of gedunin and alpha-fetoprotein showing best pose and interactions.

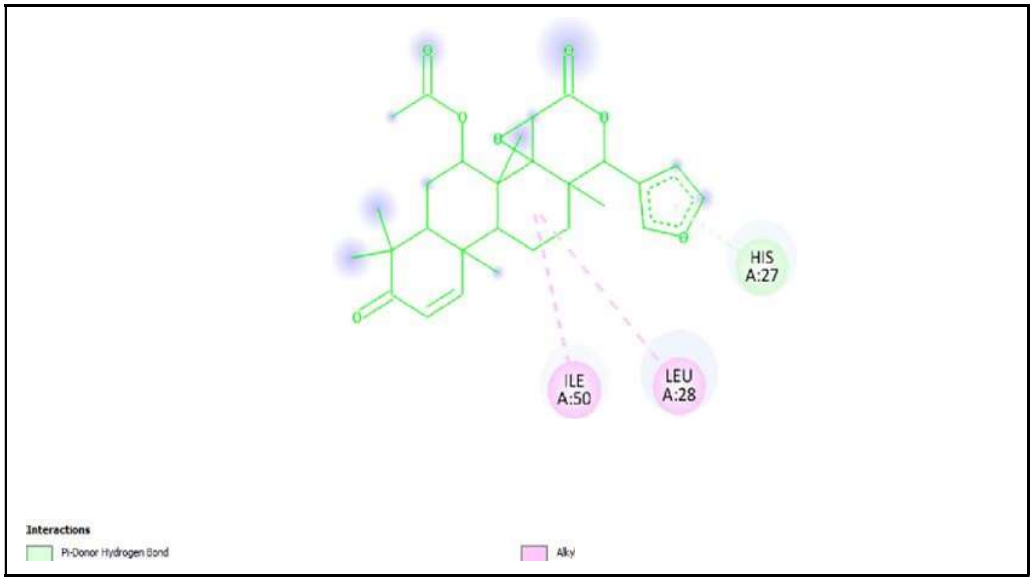


Figure 9.2. Interactions between gedunin and receptor marker 2QSQ, where pink represents alkyl bonds, whereas green represents hydrogen bonds.

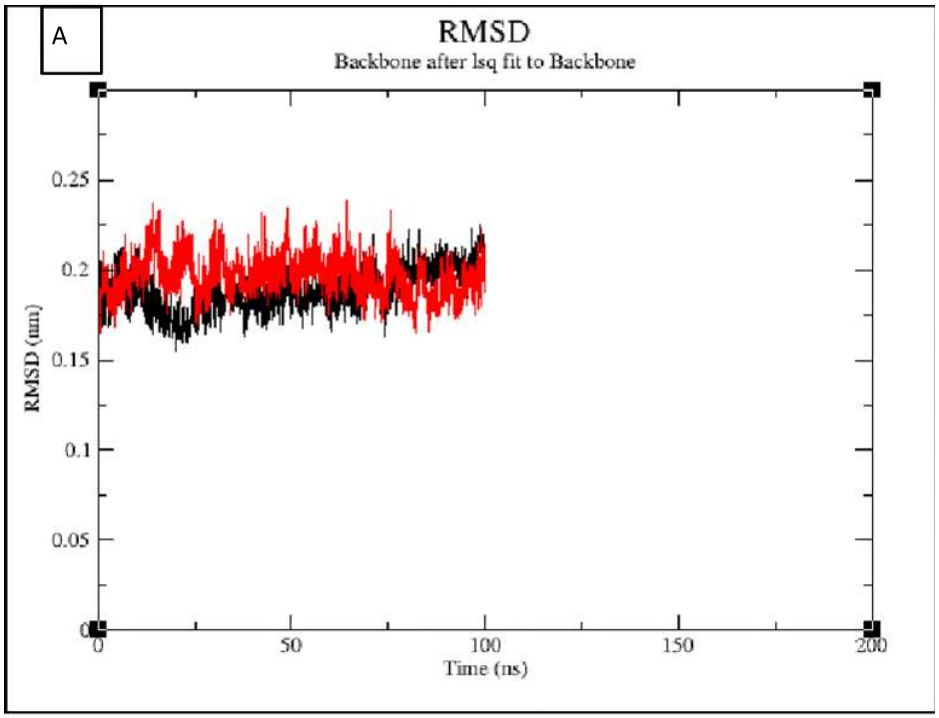
Res	#	ΔG^{FC}	$\Delta\Delta G^{R}$	ΔSASA	ΔSASA%	SASA	Cons	Rate
PHE	29	-3.12	1.98	65.67	40.00	46.79	0.64	2.05
ILE	91	-2.54	-0.48	55.94	40.50	0.00	1.00	0.39
LEU	95	-3.89	0.51	113.08	80.10	0.79	1.00	0.55

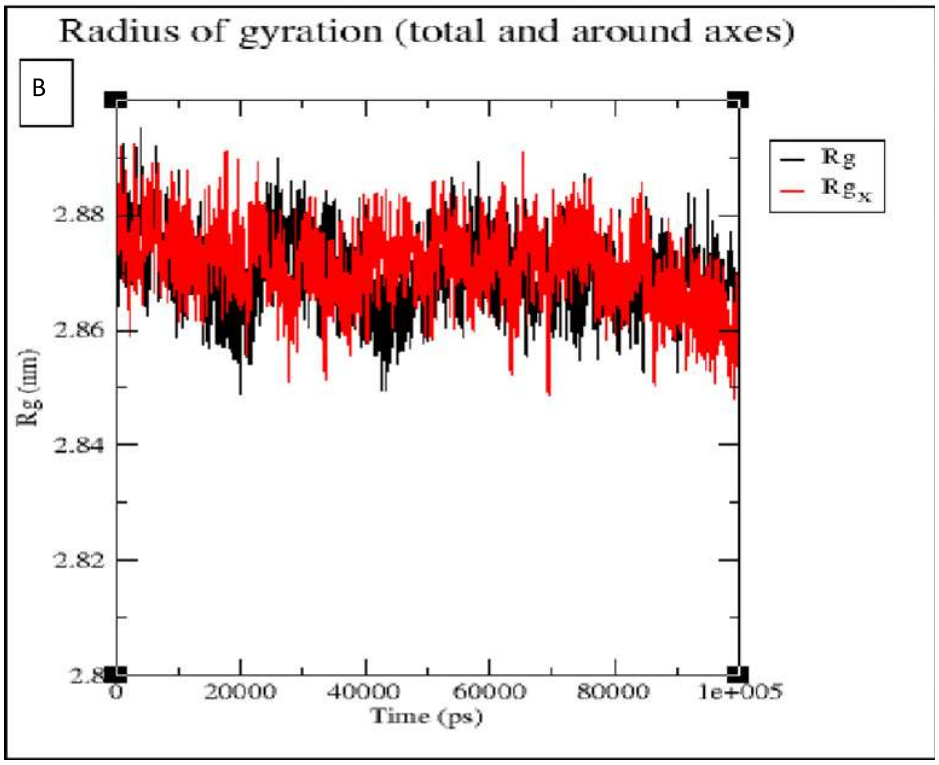
Figure 9.3. Depicts the active site of the 2QSQ receptor marker.

9.3.2. Simulation

Simulation studies of 100 ns showed that the average density between 6M4R and gedunin was 970-975 kg / m3; similarly, the average density between CEA and gedunin was 980-984 kg / m3. The radius of gyration decreased from 2.88 to 2.86 in 6M4R and gedunin, whereas 1.45 to 1.4 in CEA and gedunin after complex formation. The maximum number of hydrogen bonds between the peptide and water molecules were 2 and 1, respectively. The binding energies decreased considerably to ~ 0 Ki/mol, indicating structural stability. RMSD of ligand fluctuates from 0.2 to 0.15 nm and 0.1 to 0.2 nm, whereas rmsd of 6M4R and CEA remains stable below 0.2 nm. The Sasa (solvent accessible surface area) area was 330-320 m^2 and 72 $m^{2,}$ respectively, after docking. Overall simulation

analysis showed that gedunin bound to the active site of 6M4R and to the allosteric site of CEA, and the complex became stable **(Figure 9.4., 9.5.).**


A
RMSD
Backbone after lsq fit to Backbone
RMSD (nm)
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
Time (ns)


Radius of gyration (total and around axes)
B
Rg
Rg_x
Rg (nm)
2.88
2.86
2.84
2.82
2.8
0
20000
40000
60000
80000
1e+005
Time (ps)

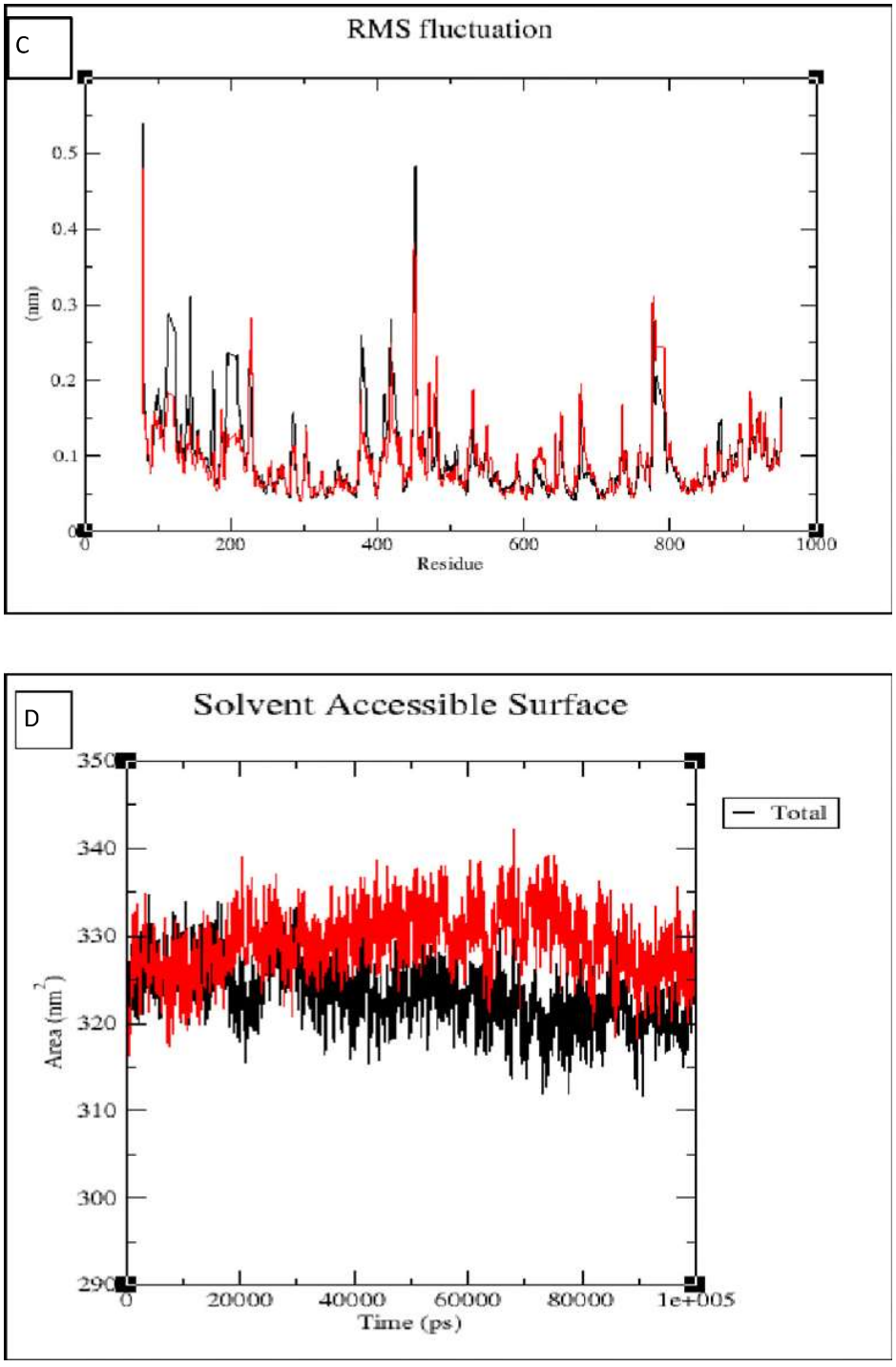
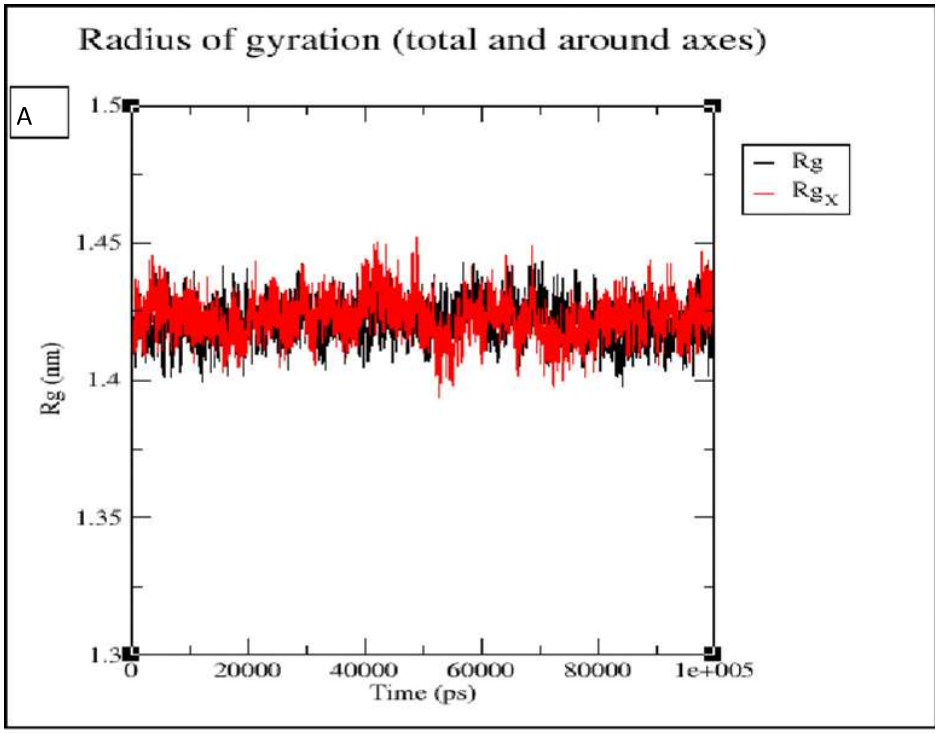
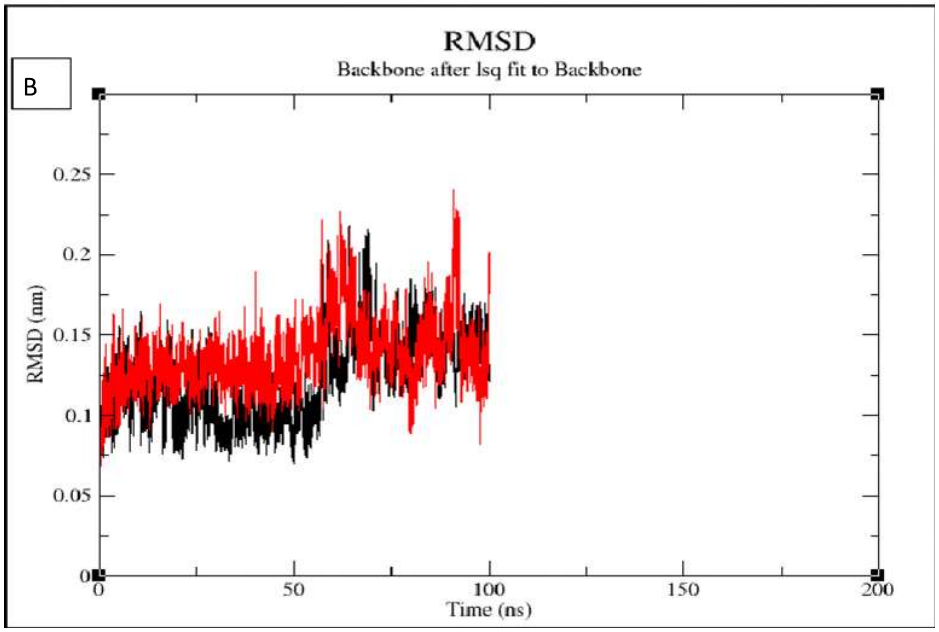


Figure 9.4. Simulation of docked gedunin and alpha feto protein recepto showing A) rmsd, (B) radius of gyration, (C) rms (root mean square fluctuations) and (D) sasa (solvent accessible surface area) where red is apo protein before docking and black is alpha feto protein after docking.


Radius of gyration (total and around axes)
A
1.5
1.45
1.4
1.35
1.3
Rg (nm)
0
20000
40000
60000
80000
1e+005
Time (ps)
Rg
Rg_X


RMSD
Backbone after lsq fit to Backbone
B
0.25
0.2
0.15
0.1
0.05
0
RMSD (nm)
0
50
100
150
200
Time (ns)

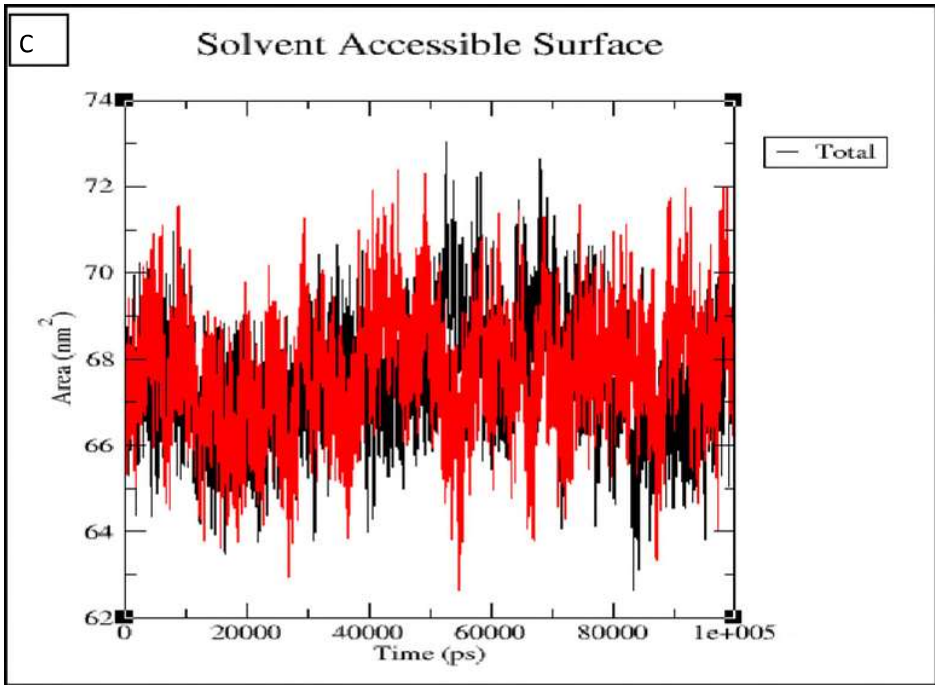


Figure 9.5. Simulation of docked gedunin and CEA receptor showing rmsd, the radius of gyration, and sasa (solvent accessible surface area) where black is apo protein CEA before docking and red is CEA after docking.

9.3.3. MTT analysis of gedunin

Figure 9.6., 9.7., 9.8., 9.9. describes the MTT assay results of HepG2 , PA1 cell line and PC3 cell line, where five concentrations were considered, namely 3.125 µg/ml (6.47mM), 6.25 µg/ml (13.72mM), 12.5 µg/ml (25.90mM), 25 µg/ml (51.80mM), and 50µg/ml (103.61mM) for HepG2 cell line and 12.5 µg/ml (25.90mM), 25µg/ml (51.80mM), 100µg/ml (207.22mM), 200µg/ml (414.44mM) for PA1 and PC3 cell line **(Figure 9.7., 9.8., 9.9).** The $_{IC50}$ of gedunin reached 35.95 µg/ml (74.49mM) in HepG2 cells after 24 hours, whereas the $_{IC50}$ of gedunin reaches to 73.71 µg/ml (152.74mM) in PA1 cells after 24 hours of incubation whereas 95.55 µg/ml (198.00mM) was observed with PC3 cell line. The graph in **Figure 9.10., 9.11.** showed the accuracies of 0.977 R^2 for PC3 cells

and 0.865 R^2 for PA1 cell lines.**Table 9.1.** showed the graph with an accuracy of 0.989 R^2 in HepG2 cell line, where the x-axis is the concentration in µg/ml, and the y-axis is the percentage viability of cells **(Uchil *et al* ., 2018)**.

Table 9.1. MTT Data Analysis-HepG2 cell line with Gedunin treatment.

			Concentrations (in µg/mL)				
	Blank	Untreated	3.125	6.25	12.5	25	50
Reading 1	0.008	1.568	1.22	1.143	1.106	0.901	0.606
Reading 2	0.007	1.595	1.173	1.164	1.168	0.973	0.607
Mean OD	0.008	1.582	1.197	1.154	1.137	0.937	0.607
Mean OD-Mean Blank		1.5740	1.1890	1.1460	1.1295	0.9295	0.5990
Standard deviation		0.0191	0.0332	0.0148	0.0438	0.0509	0.0007
Standard error		0.0135	0.0235	0.0105	0.0310	0.0360	0.0005
% Standard error		0.8577	1.4930	0.6671	1.9695	2.2872	0.0318
% Viability		100	75.54	72.81	71.76	59.05	38.06
IC_{50} = 35.95 µg/ml							

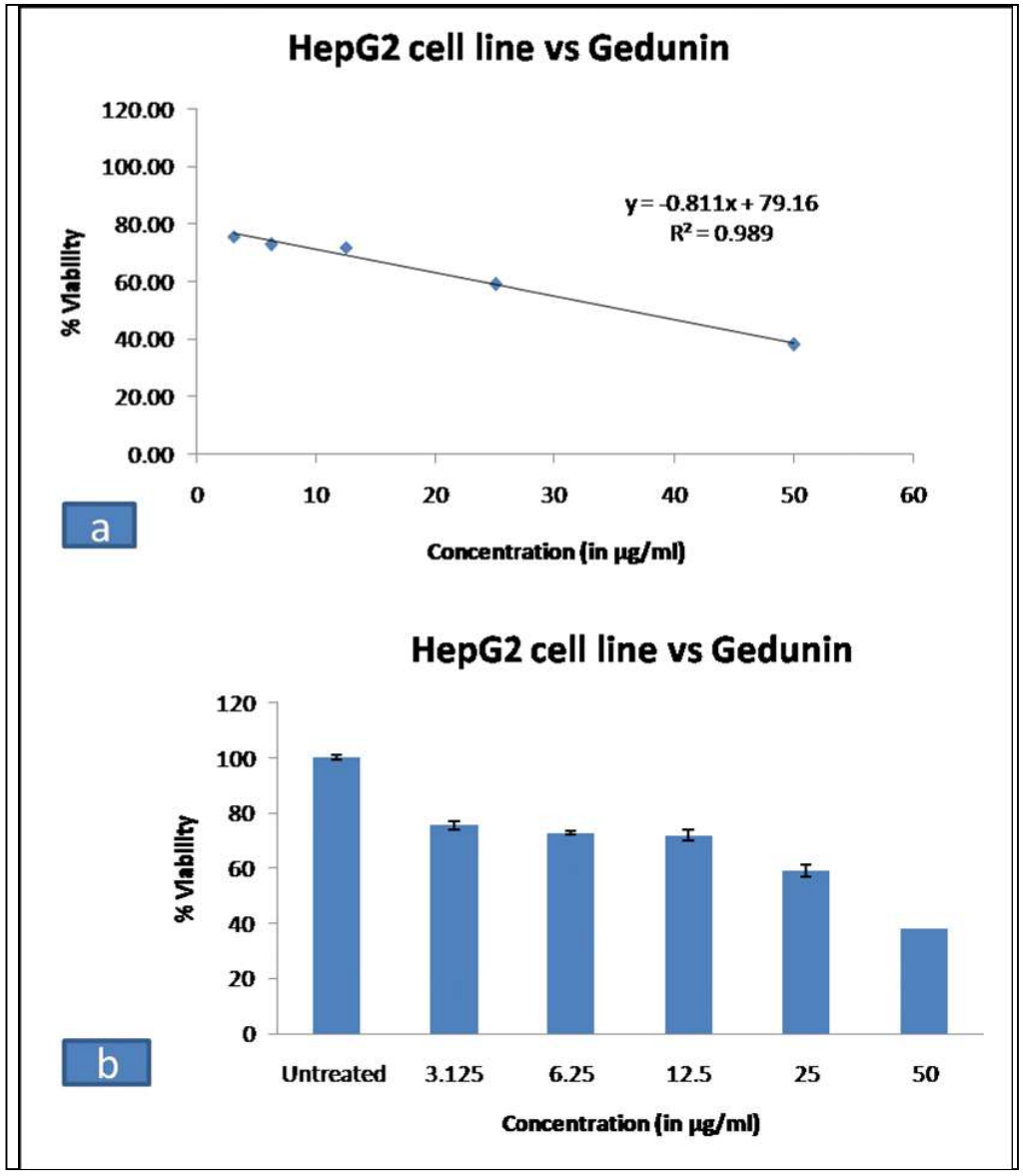
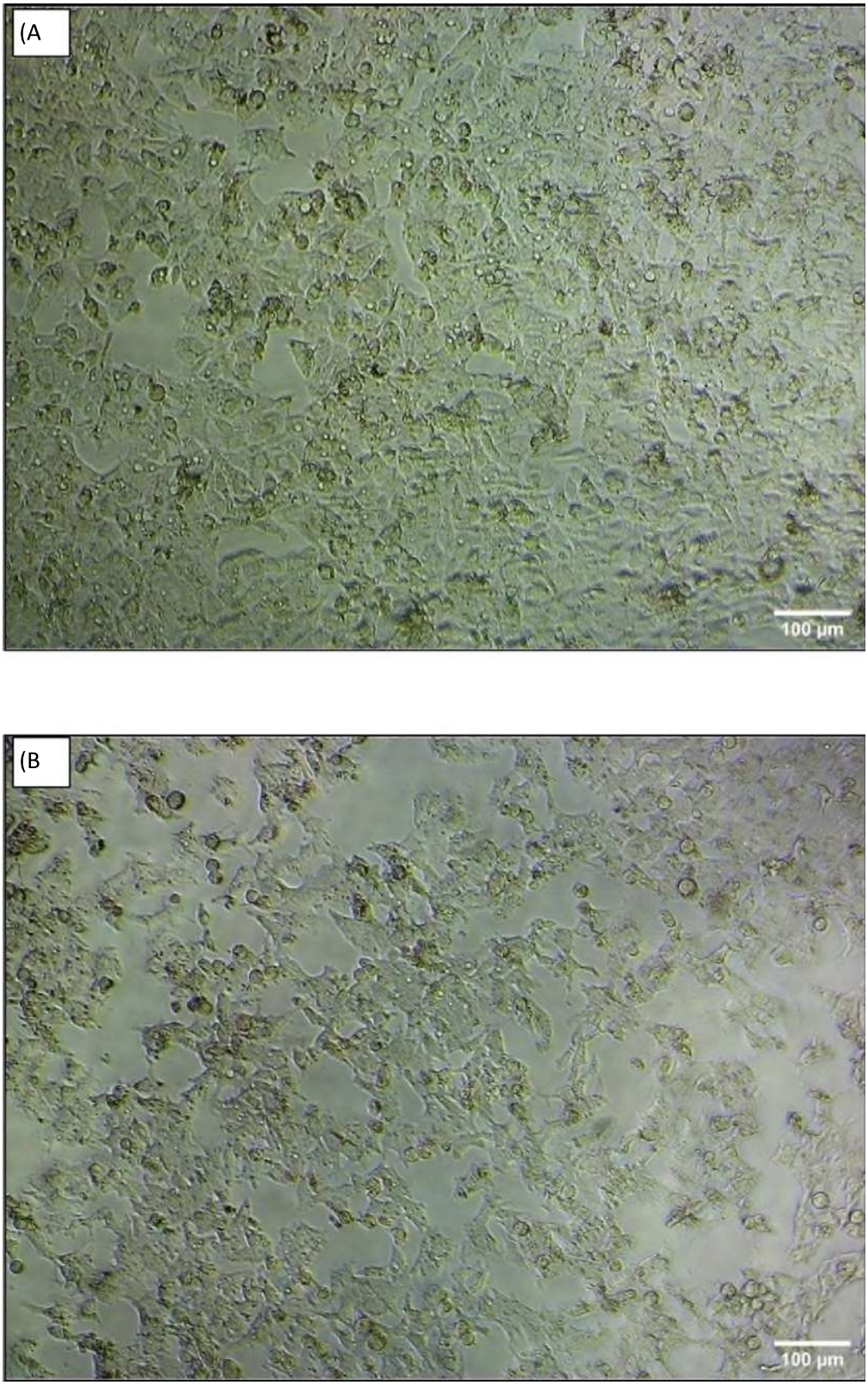
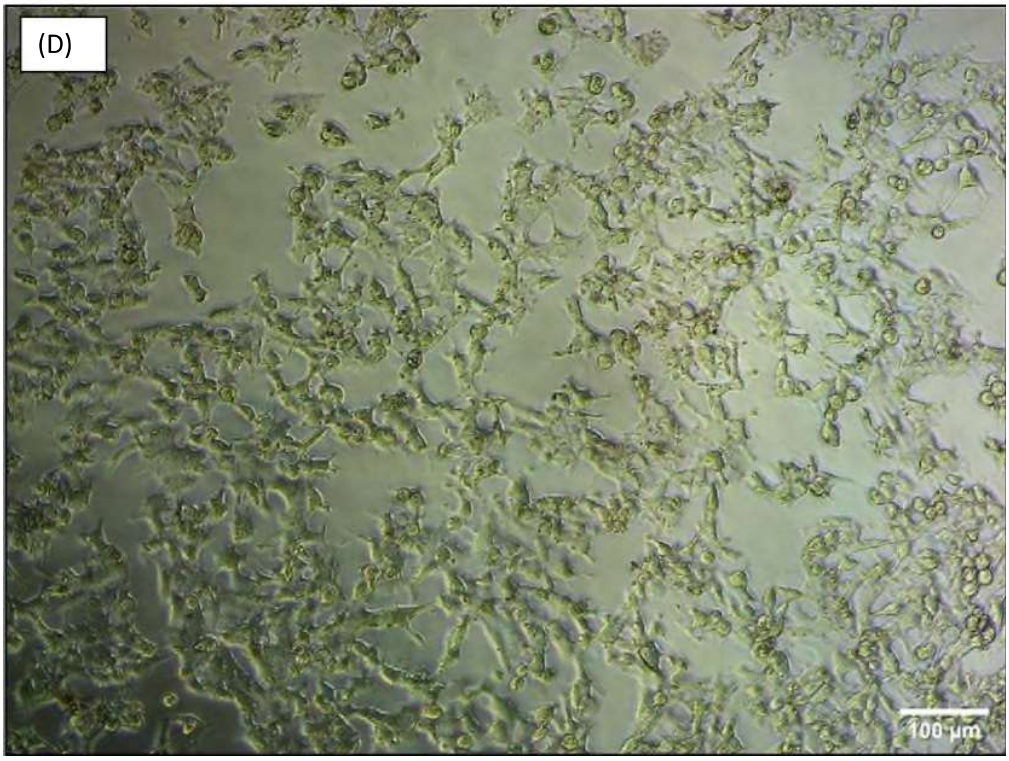


Figure 9.6. (a) It shows that the percent viability of the HepG2 cell line decreases as the concentration increases, and the R^2 of the graph is 0.989, (b) It depicts the five concentrations of the MTT assay, where $_{IC50}$ comes at a concentration of 35.95 µg/ml. The five concentrations are 3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, and 50 µg/ml.


(A
100 µm
(B
100 µm


(C)
100 µm


(D)
100 µm

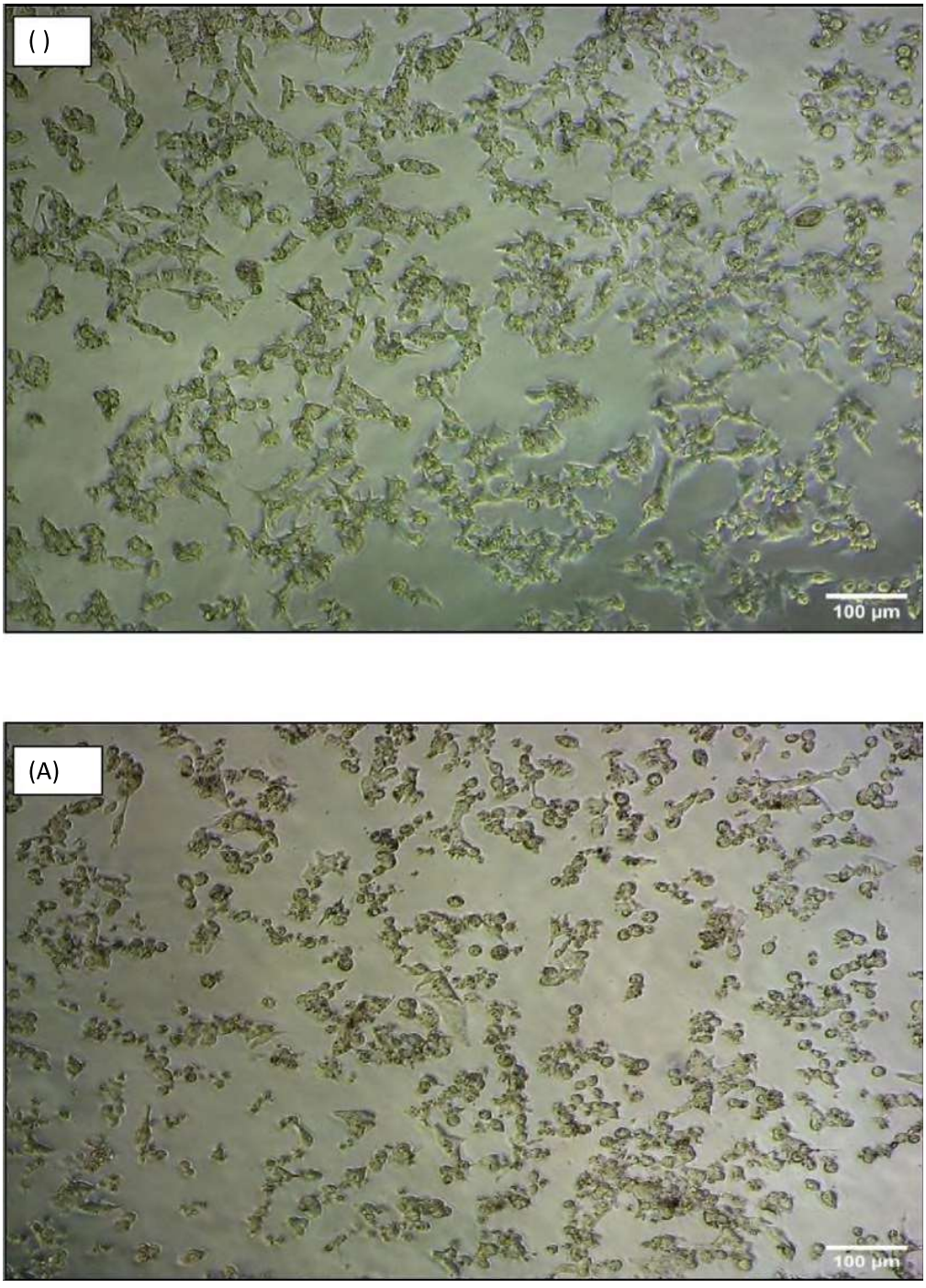
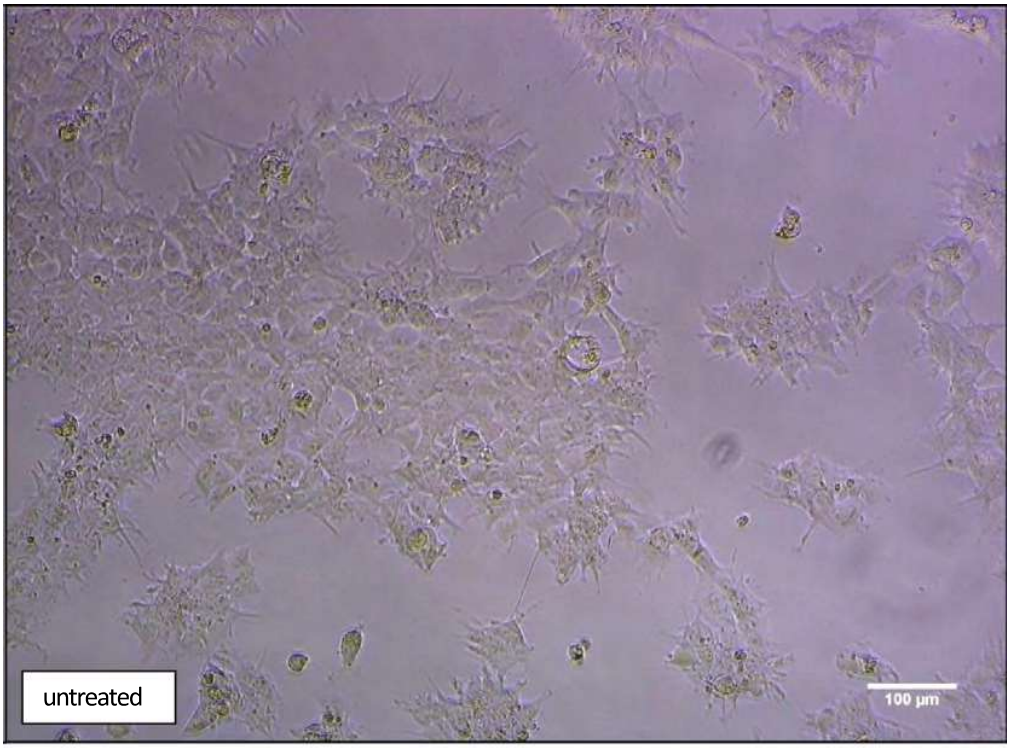
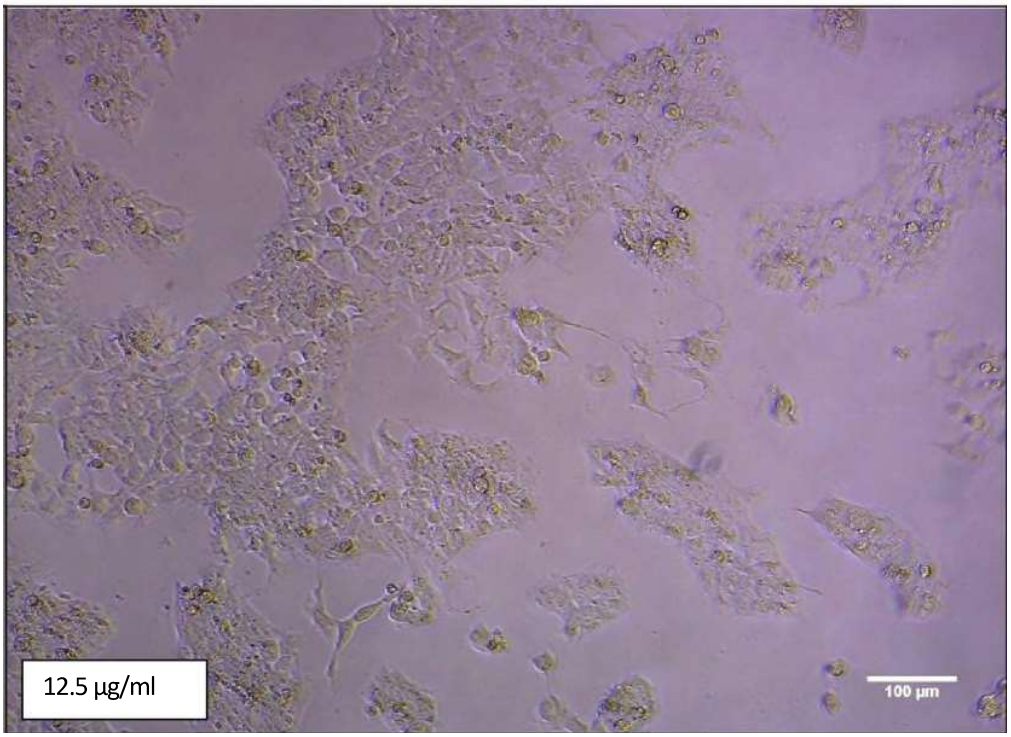
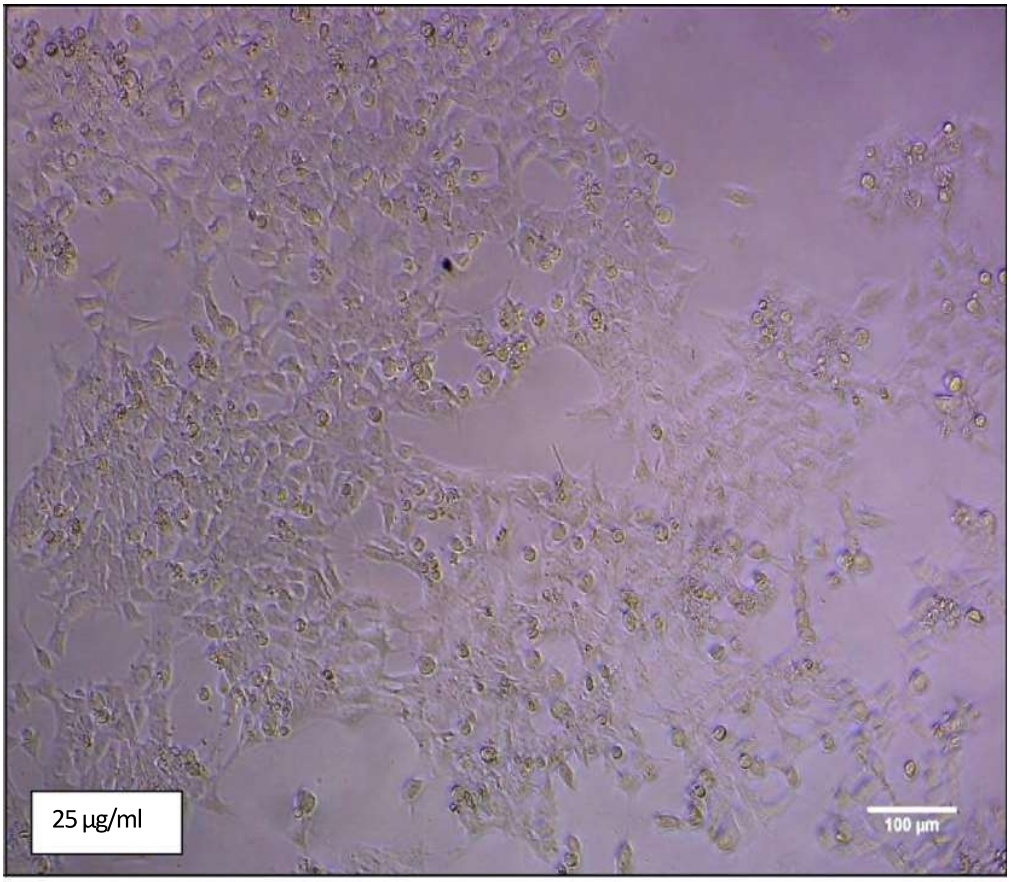
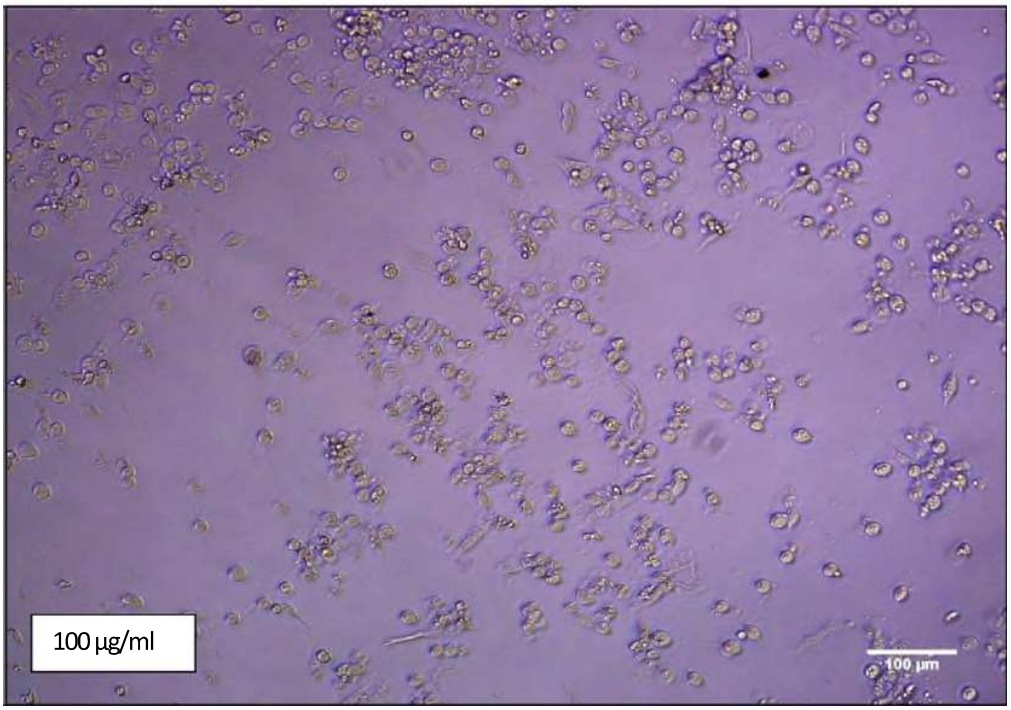


Figure 9.7. HepG2 cell viability in presence of gedunin at different concentrations (A) Untreated, (B) 3.125 µg/ml, (C) 6.25 µg/ml, (D) 12.5 µg/ml, (E) 25µg/ml (F) 50 µg/ml with the resolution of 100 µm.


untreated
100 µm


12.5 µg/ml
100 µm


25 µg/ml
100 µm


100 µg/ml
100 µm

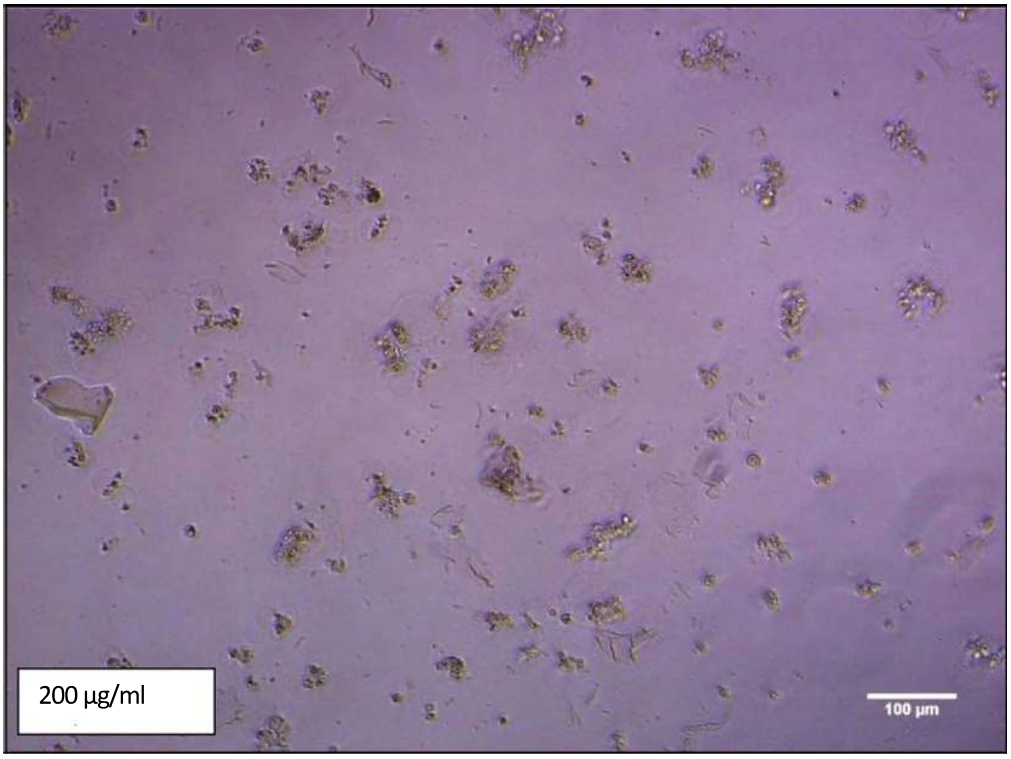
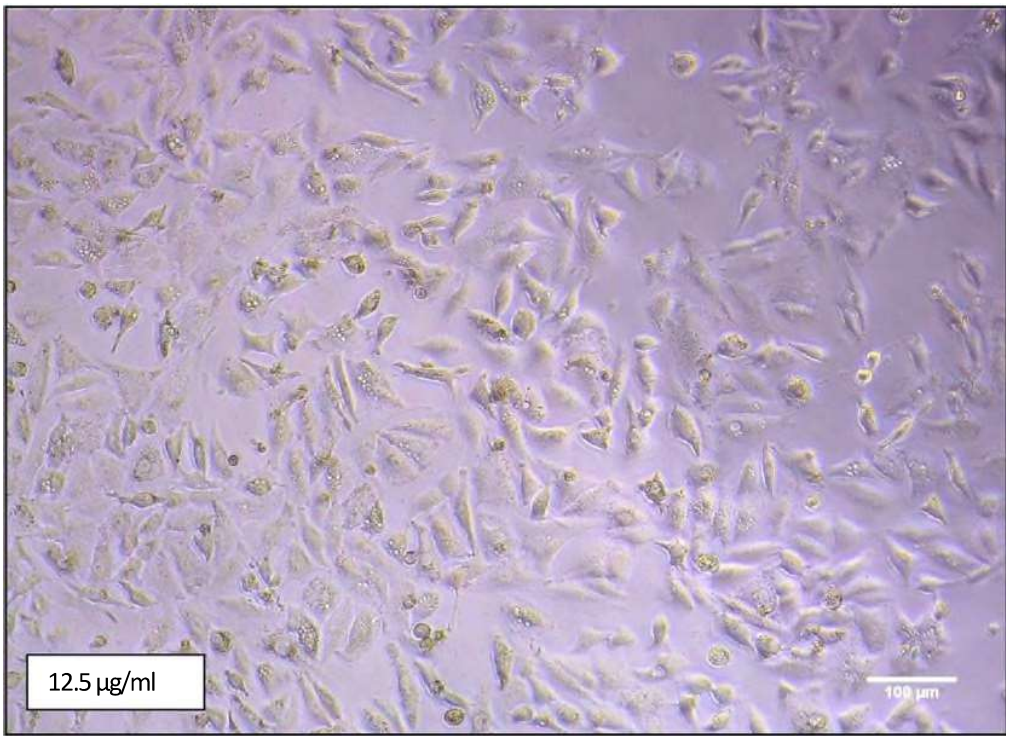
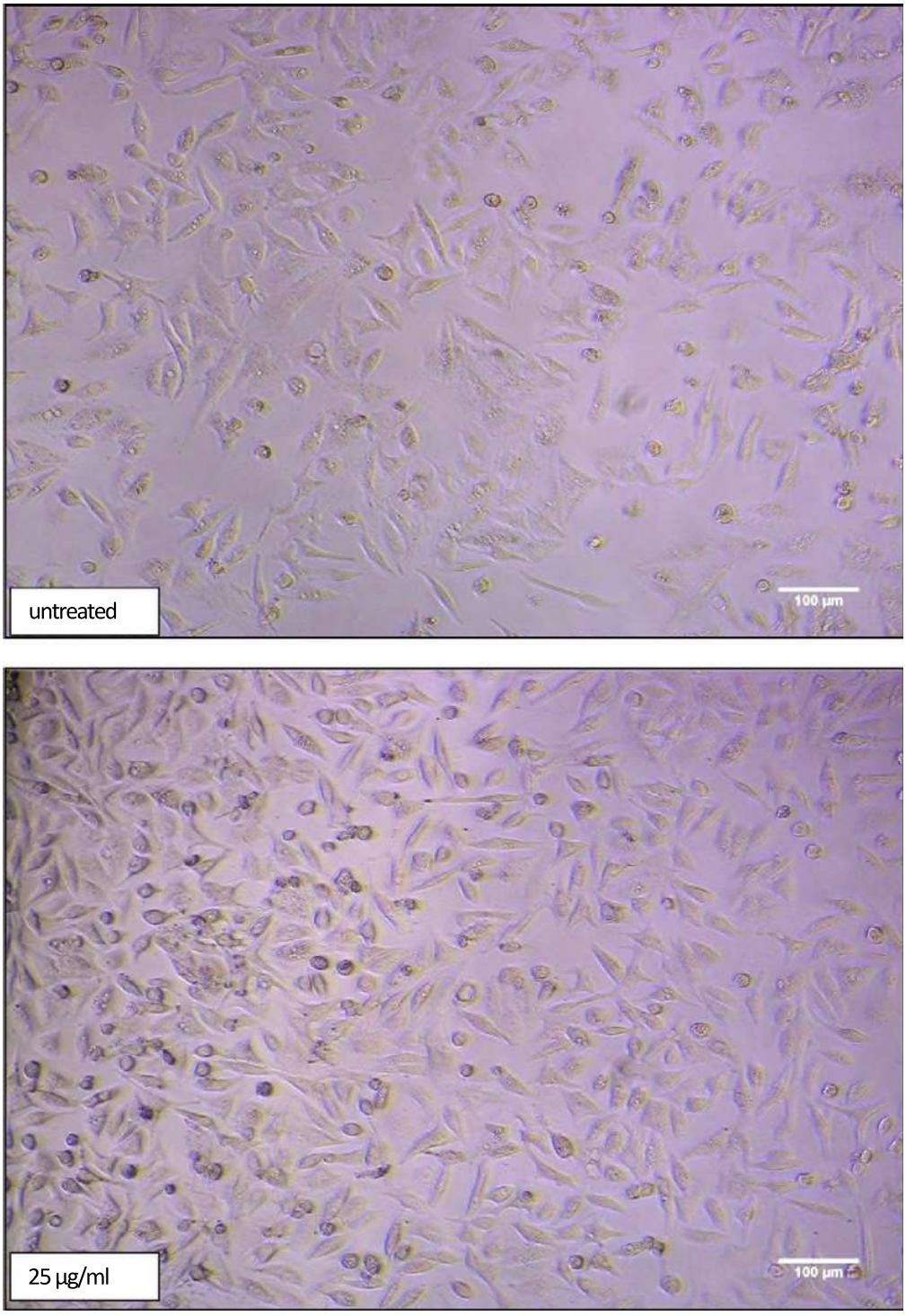


Figure 9.8. Cytotoxicity assay of PA1 cells with concentrations of 12.5 µg/ml, 25µg/ml, 100 µg/ml, 200 µg/ml of gedunin at the resolution of



100µm.


untreated
100 µm
25 µg/ml
100 µm

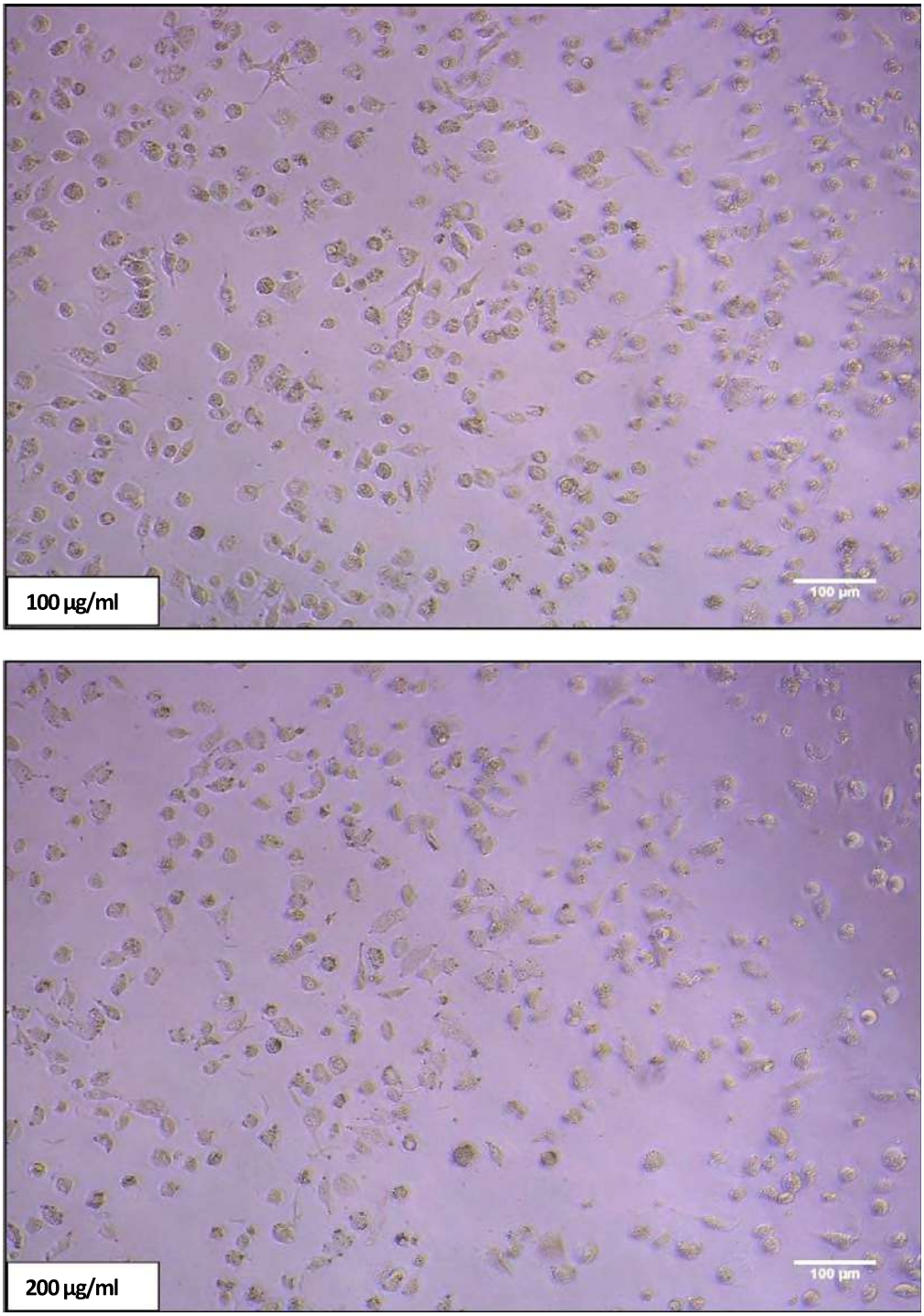
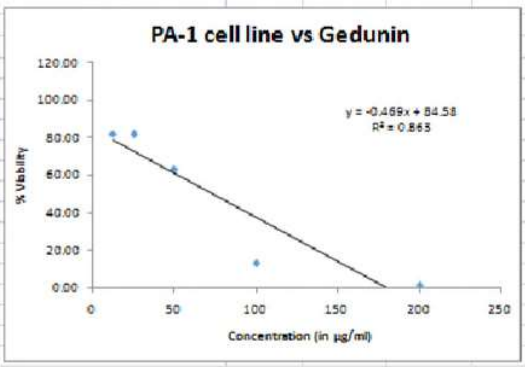


Figure 9.9. Cytotoxicity assay of PC3 cells with concentration of 12.5 µg/ml,25µg/ml,100 µg/ml, 200 µg/ml of gedunin at the resolution of 100µm.

MTT Data Analysis- PA-1 cell line vs Gedunin

			Test concentrations (µg/ml)				
	Blank	Untreated	12.5	25	50	100	200
Reading 1	0.002	0.934	0.793	0.801	0.608	0.129	0.017
Reading 2	0.002	0.998	0.794	0.781	0.607	0.127	0.012
Mean OD	0.002	0.966	0.794	0.791	0.608	0.128	0.015
Mean OD-Mean Blank		0.9640	0.7915	0.7890	0.6055	0.1260	0.0125
Standard deviation		0.0453	0.0007	0.0141	0.0007	0.0014	0.0035
Standard error		0.0320	0.0005	0.0100	0.0005	0.0010	0.0025
% Standard error		3.3195	0.0519	1.0373	0.0519	0.1037	0.2593
% Viability		100	82.11	81.85	62.81	13.07	1.30

IC50 = 73.71µg/ml



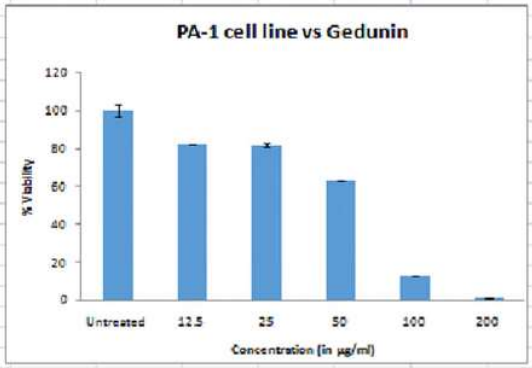
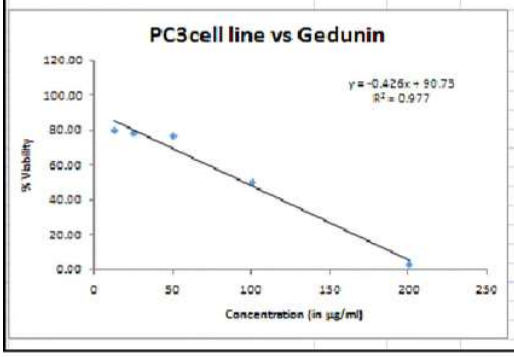


Figure 9.10. MTT data analysis of PA1 cell line on gedunin treatment.

MTT Data Analysis - PC3 cell line vs Gedunin

PC3 cell line vs Gedunin			Test concentrations (µg/ml)				
	Blank	Untreated	12.5	25	50	100	200
Reading 1	0.002	1.328	1.062	1.027	1.032	0.685	0.041
Reading 2	0.002	1.322	1.063	1.042	1.007	0.649	0.045
Mean OD	0.002	1.325	1.063	1.035	1.020	0.667	0.043
Mean OD-Mean Blank		1.3230	1.0605	1.0325	1.0175	0.6650	0.0410
Standard deviation		0.0042	0.0007	0.0106	0.0177	0.0255	0.0028
Standard error		0.0030	0.0005	0.0075	0.0125	0.0180	0.0020
% Standard error		0.2268	0.0378	0.5669	0.9448	1.3605	0.1512
% Viability		100	80.16	78.04	76.91	50.26	3.10

IC50 = 95.55µg/ml



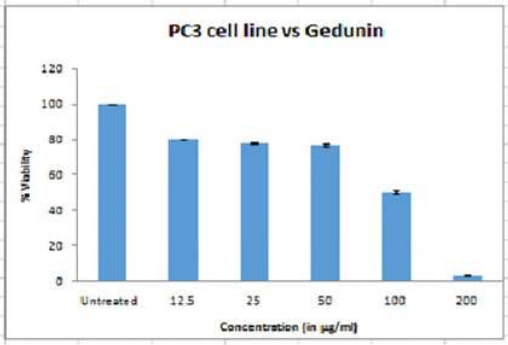


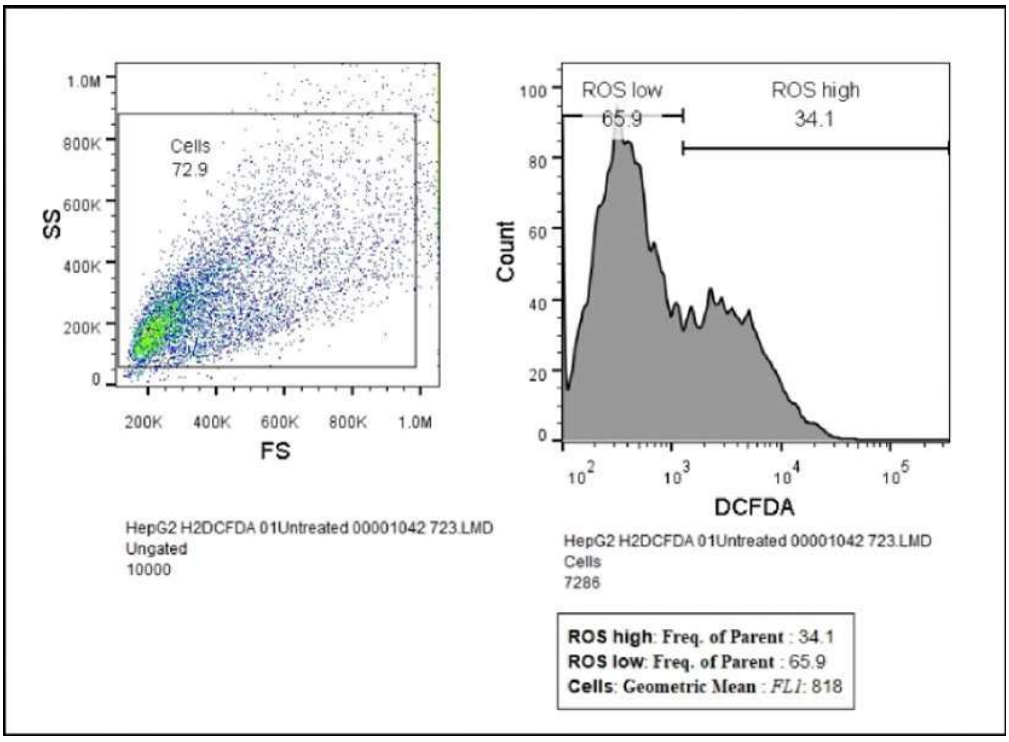
Figure 9.11. MTT data analysis of PC3 cell line on gedunin treatment.

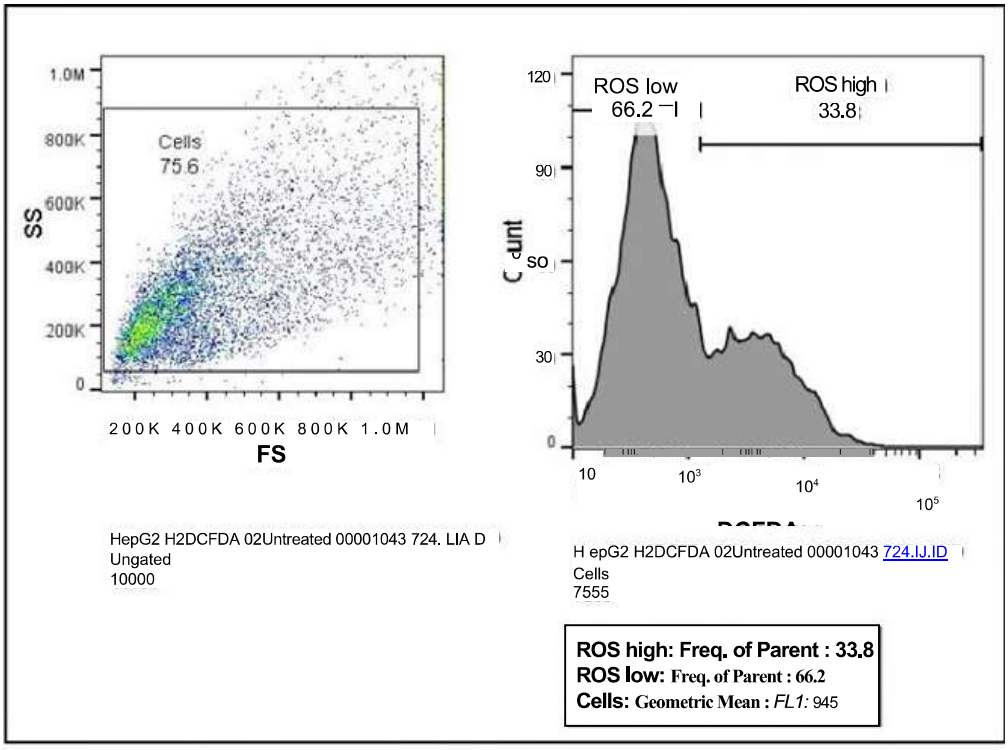
9.3.4. Evaluation of ROS activity assay using HepG2 cell line

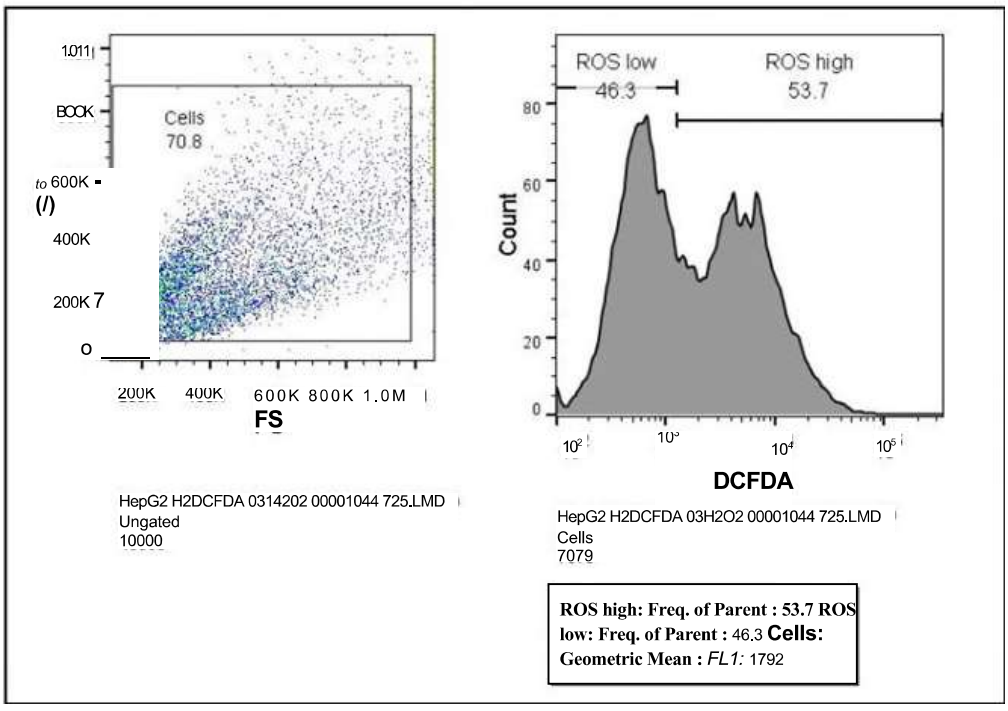
Reactive oxygen species (ROS) are hydroxyl radicals or unpaired electron peroxide molecules. ROS are generated spontaneously in healthy aerobic cells as a by-product of oxidative phosphorylation, a regulated rate of oxidoreductase enzymes, or a metal-catalyzed oxide. However, ROS can increase exposure to environmental oxidants and certain medications, contributing to oxidative stress under specific conditions. Over-ROS may harm the building blocks of cells, such as DNA, proteins, and lipids, ultimately resulting in cell mortality.2', 7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) is an indicator used by many cell permeants **(Horvath *et al.,* 2015)**. The decreased fluorescein H_2DCFDA may be oxidized and transformed into intracellular ROS to fluorescent fluorescein 2,' 7,'-dichlorofluorescein (DCF). In this procedure, intracellular ROS was labeled with H_2DCFDA, and DCF intensity was measured by flow cytometry. H_2DCFDA is oxidized and intracellularly transformed to 2', 7'-dichlorofluorescein (DCF). Cells with high ROS levels were thus vividly green and exhibited a high MFI level of DCFDA (FL1-A parameter) **(Table 9.2.).** Gedunin (nontoxic concentration)-pretreated cells with the test sample 24 hours before therapy for H_2O_2 exhibited lower ROS levels than untreated cells. Treatment of cells with H_2O_2 resulted in the highest ROS levels. A filter 525 nm band pass filter was used to collect FITC fluorescence (green) in an FL1 detector. **(Figure 9.12, 9.13.)**

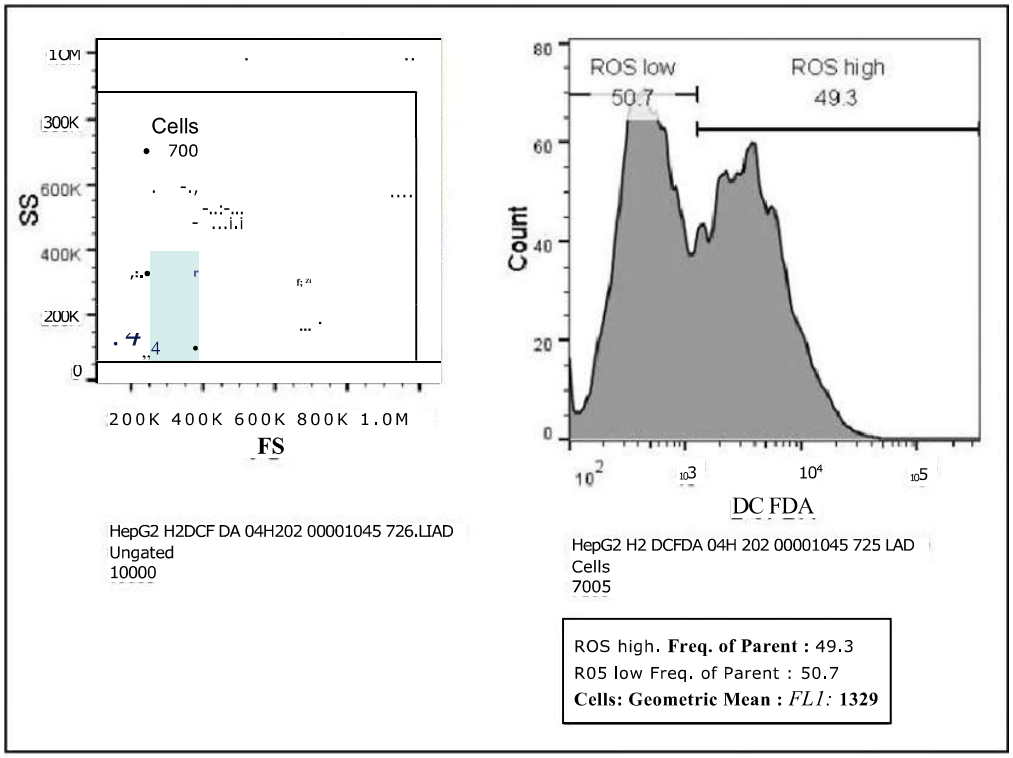
Table 9.2. ROS activity of gedunin using HepG2 cells.

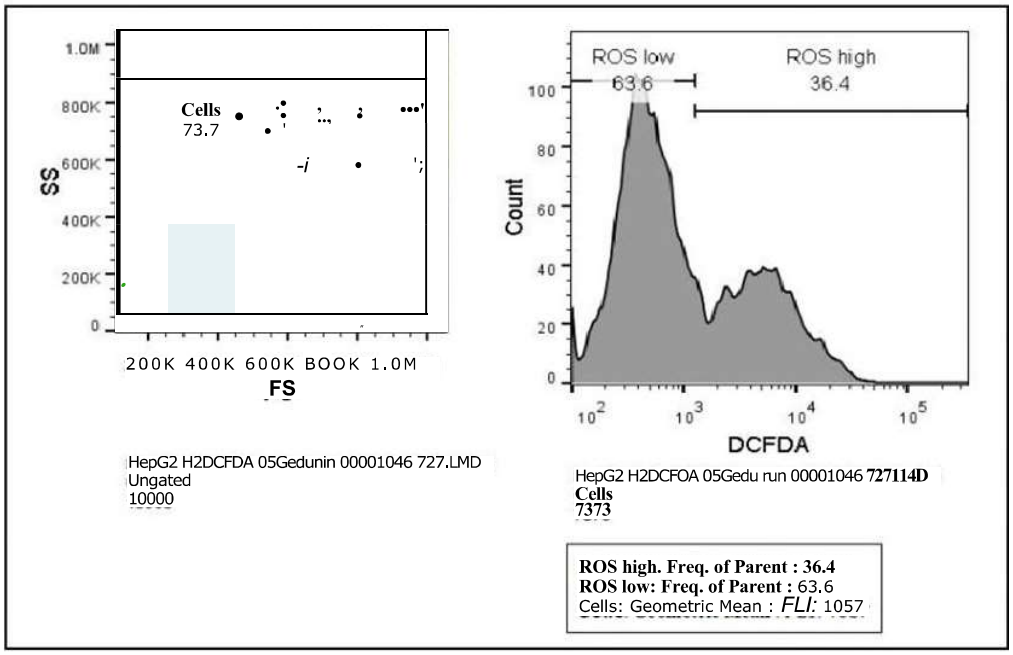
S.No.	Sample Name	% of Cells		Geometric mean fluorescence intensity (MFI) of DFCDA
		ROS Low	ROS High	
1	**Untreated**	65.9	34.1	818
2	**Untreated**	66.2	33.8	945
3	**H_2O_2 (100µM) Induced**	46.3	53.7	1792
4	**H_2O_2 (100µM) Induced**	50.7	49.3	1320
5	**Pretreated with Sample Gedunin (3 µg/ml) or (6.21mM) + H_2O_2 (100µM)**	63.6	36.4	1057
6	**Pretreated with Sample Gedunin (3 µg/ml) or (6.21mM) + H_2O_2 (100µM) Induced**	65.6	34.4	893




1.0M
800K
600K
400K
200K
0
SS
Cells
75.6
200K 400K 600K 800K 1.0M
FS
120
90
60
30
0
Count
ROS low
66.2
ROS high
33.8
10
10³
10⁴
10⁵
HepG2 H2DCFDA 02Untreated 00001043 724. LIA D
Ungated
10000
H epG2 H2DCFDA 02Untreated 00001043 724.IJ.ID
Cells
7555
ROS high: Freq. of Parent : 33.8
ROS low: Freq. of Parent : 66.2
Cells: Geometric Mean : FL1: 945


1.011
800K
600K
400K
200K
0
Cells
70.8
200K 400K 600K 800K 1.0M
FS
80
60
40
20
0
Count
ROS low
46.3
ROS high
53.7
10²
10³
10⁴
10⁵
DCFDA
HepG2 H2DCFDA 0314202 00001044 725.LMD
Ungated
10000
HepG2 H2DCFDA 03H2O2 00001044 725.LMD
Cells
7079
ROS high: Freq. of Parent : 53.7 ROS low: Freq. of Parent : 46.3 Cells: Geometric Mean : FL1: 1792


Cells
700
SS
FS
200K 400K 600K 800K 1.0M
ROS low
50.7
ROS high
49.3
Count
DC FDA
HepG2 H2DCF DA 04H202 00001045 726.LIAD
Ungated
10000
HepG2 H2 DCFDA 04H 202 00001045 725 LAD
Cells
7005
ROS high. Freq. of Parent : 49.3
R05 low Freq. of Parent : 50.7
Cells: Geometric Mean : FL1: 1329


Cells
73.7
SS
FS
200K 400K 600K BOOK 1.0M
ROS low
63.6
ROS high
36.4
Count
DCFDA
HepG2 H2DCFDA 05Gedunin 00001046 727.LMD
Ungated
10000
HepG2 H2DCFOA 05Gedu run 00001046 727114D
Cells
7373
ROS high. Freq. of Parent : 36.4
ROS low: Freq. of Parent : 63.6
Cells: Geometric Mean : FLI: 1057

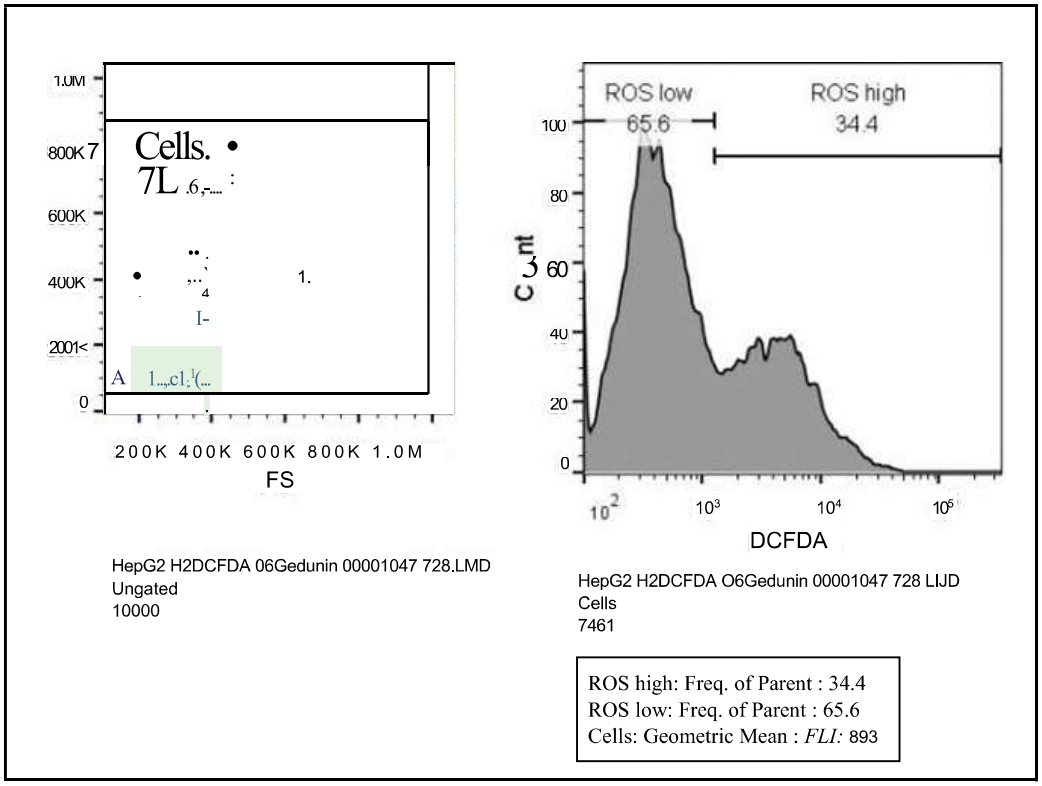


Figure 9.12. Detection of ROS activity of HepG2 cell line using flow cytometry.

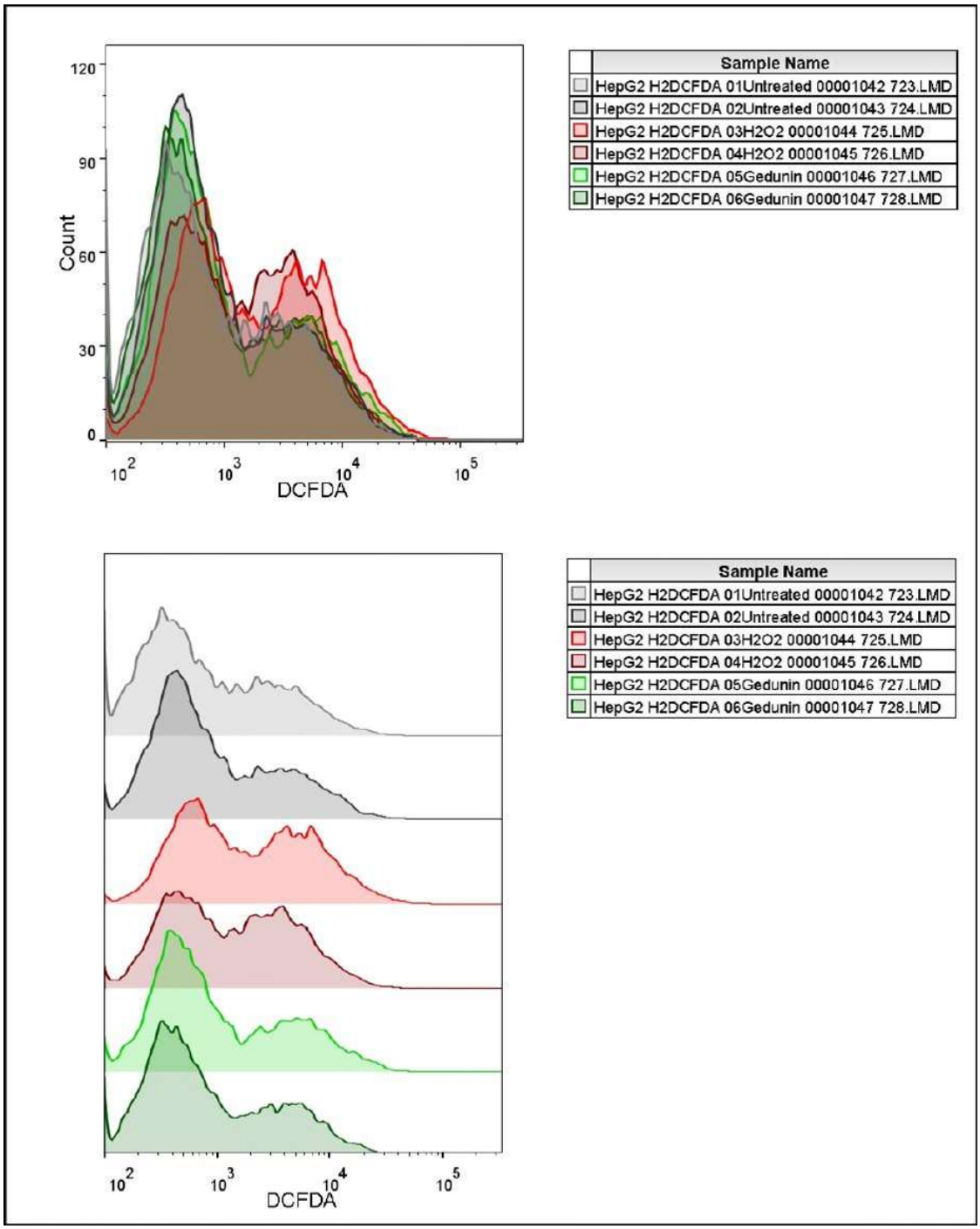


Figure 9.13. ROS activity assay of HepG2 cell line.

9.3.5. Evaluation of Apoptosis assay using HepG2 cell line, PA1 and PC3 cell line and non cancerous cell line (NIH/3t3).

Apoptosis and inflammation are significant components of the caspase family of cysteine proteases. Caspase 3 is an essential protease in the early stages of apoptosis and is generated as an inactive pro-enzyme that is self-depleting and cleaution-processed by another protease in cells subjected to apoptosis. The caspase types processed include large units (17-22 kDa) and smaller subunits (1012 kDa), producing an active enzyme. The 32 kDa pro-enzyme was used to create active caspase-3, 17, and 12 kDa subunits for apoptosis-prone cells **(Dai *et al*., 2017).** Active caspase-3 divides and activates additional caspases and key cytoplasmic targets, such as proteolytically, D4-GDI, Bcl-2, and nuclides (e.g., PARP). These anticorps have been notably utilized in human and mouse cells to detect the activated form of caspase 3. The pro-enzyme of Caspase-3 has not been reported. In contrast to the control and substantial growth in FITC, the proportion of positive cells in the gedunin sample-treated cells in the test sample increased the mean fluorescence intensity. **(Table 9.3., 9.4.).** This is equivalent to an increased expression of active caspase three after treatment with the gedunin test sample. A filter 525 nm band pass filter was used to gather FITC fluorescence (green) in an FL1 detector **(Figure 9.14, 9.15, 9.16, 9.17, and 9.18).**

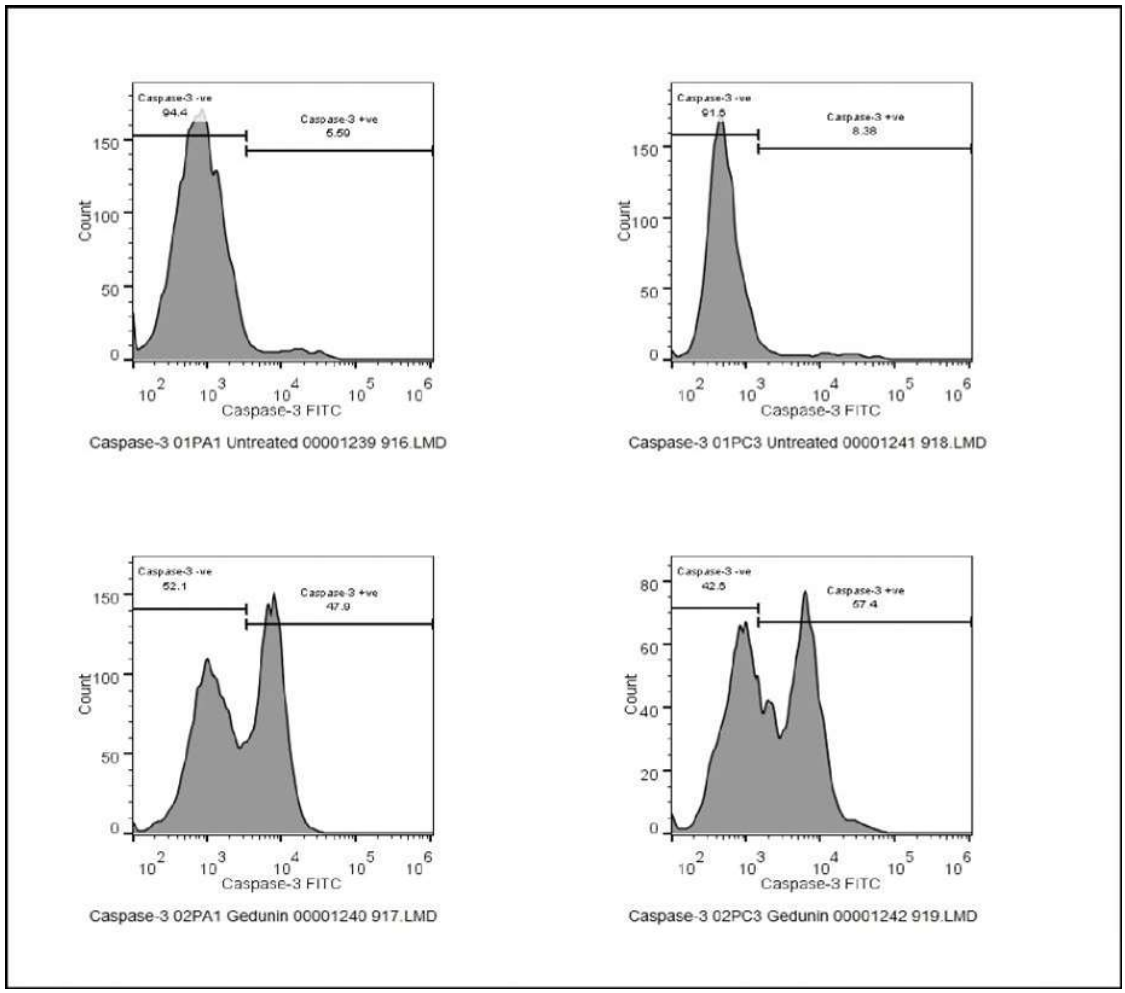


Figure 9.14. Apoptosis assay of NIH/3t3 cell line

Table 9.3. Apoptosis assay of PA1 and PC3 cell lines on gedunin treatment.

S.No.	Sample Name	Geometric mean fluorescence intensity (MFI) of FITCCaspase-3 A parameter)	Fold increase in Caspase-3 expression	% of Cells	
				Caspase-3 Low	Caspase -3 high
PA-1 cell line					
1	**Untreated**	886	1	94.4	5.6
2	**Gedunin -** 73.71 µg/mL (IC_{50})	2721	3.1	52.1	47.9
PC-3 cell line					
1	**Untreated**	623	1	91.6	8.4

2	**Gedunin** - 95.55 µg/mL (IC_{50})	2243	3.6	42.6	57.4

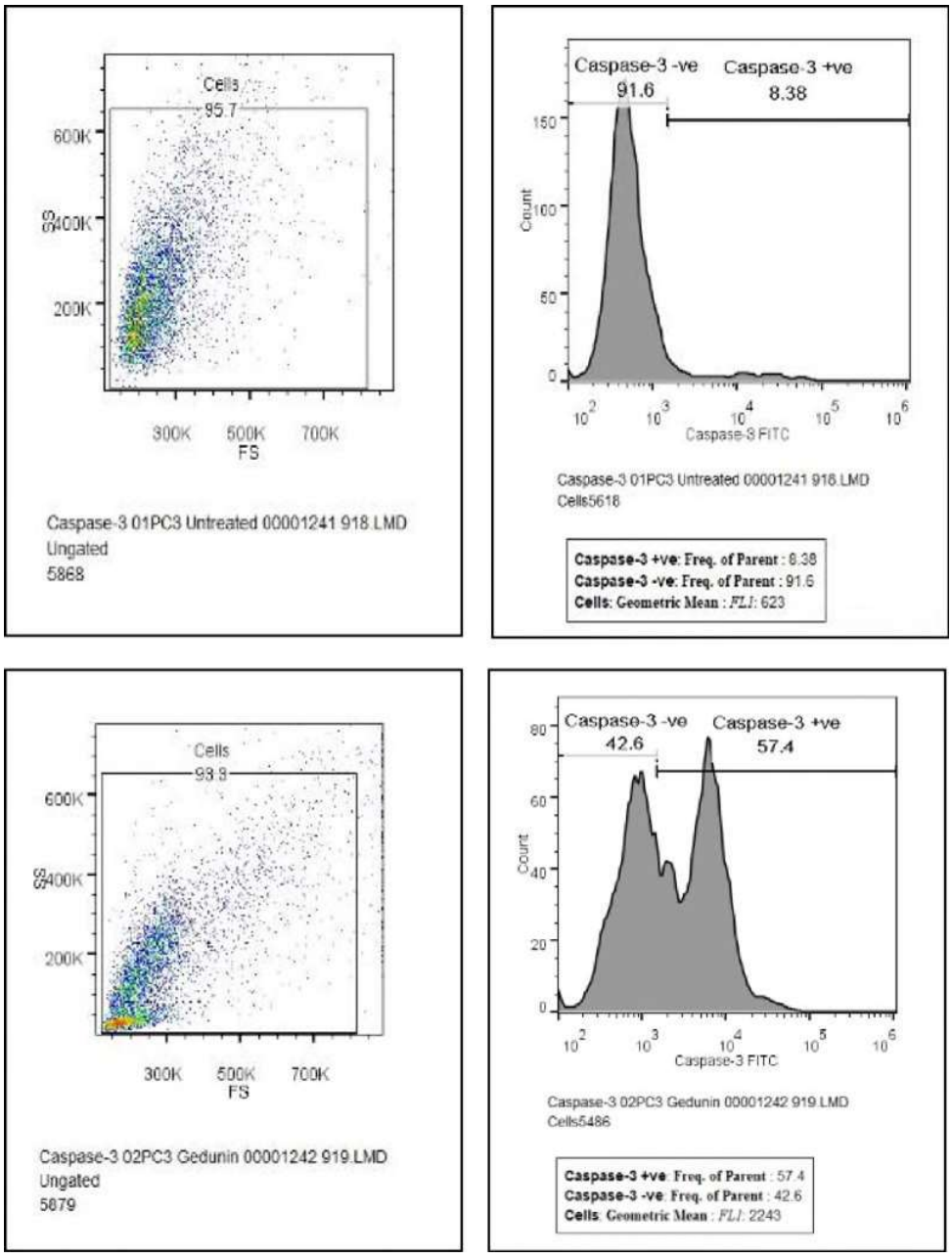


Figure 9.15. Apoptosis assay of PC3 cell line

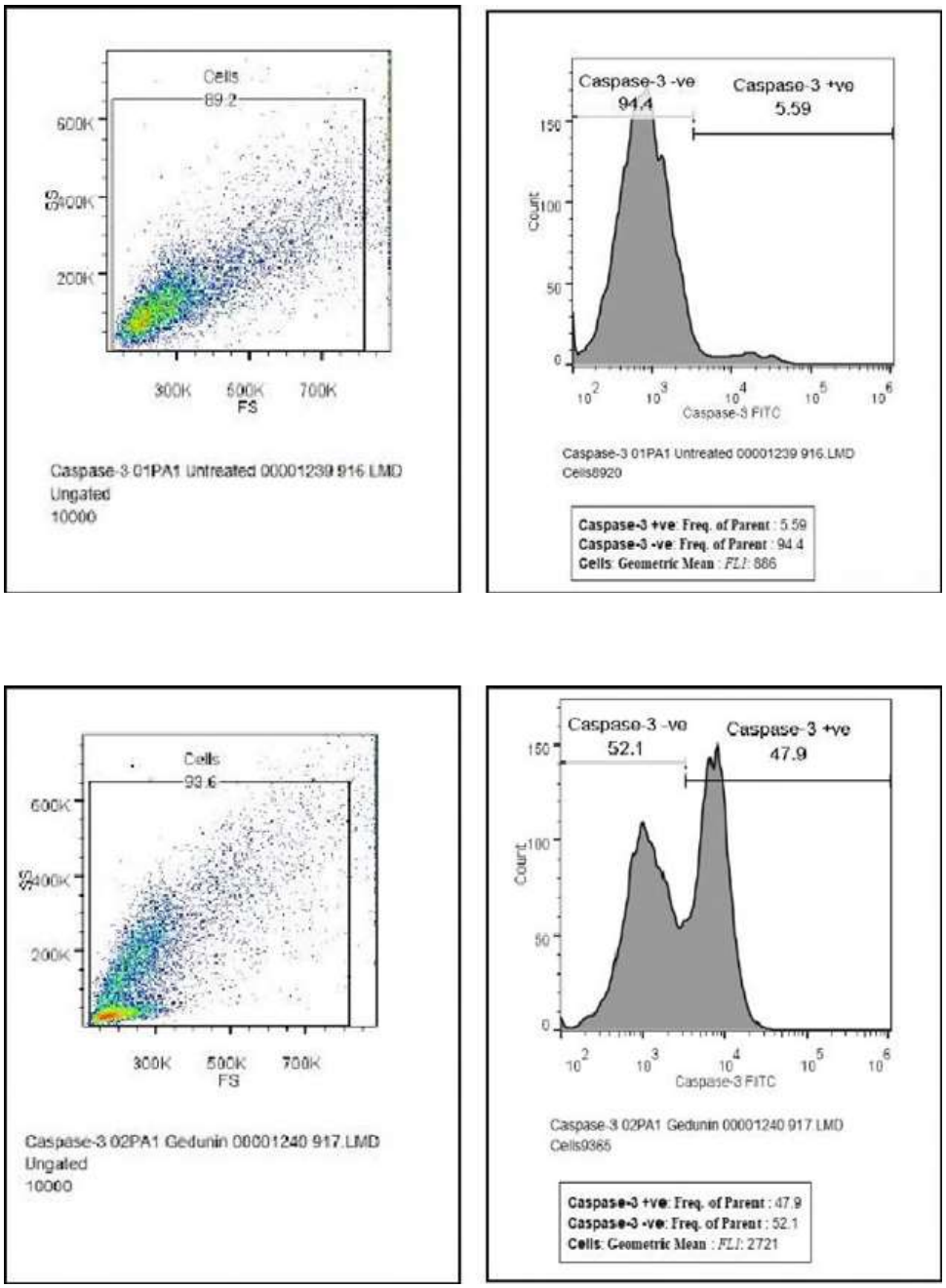
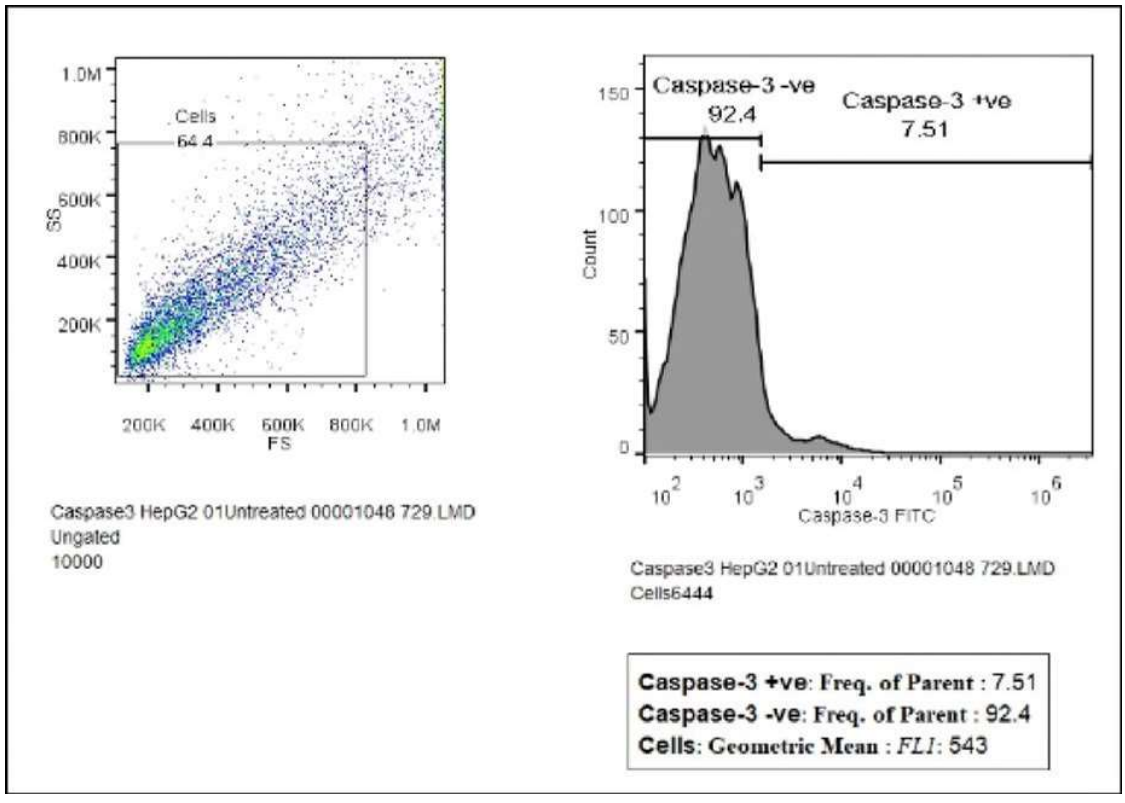
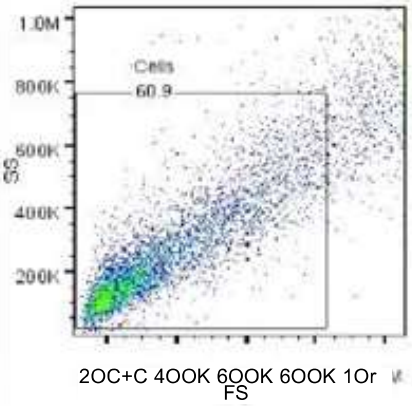


Figure 9.16. Apoptosis assay of PA1 cell line.

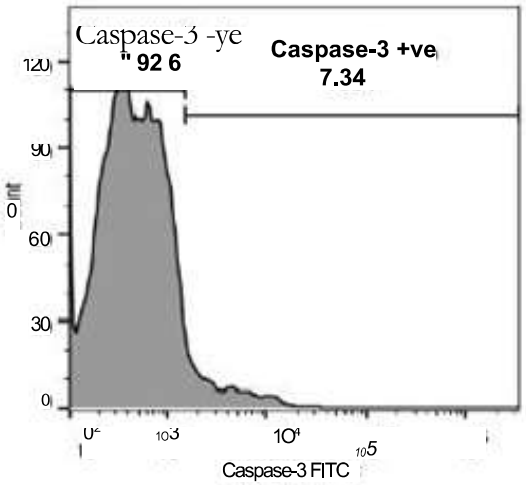
Table 9.4. Apoptosis / caspase-3 assay of gedunin using HepG2 cells.

S.No.	Sample Name	Geometric mean fluorescence intensity (MFI)	% of Cells	
			Caspase-3 -ve	Caspase-3 +ve
1	**Untreated**	of543	92.4	7.51
2	**Untreated**	Cas 490	92.6	7.34
3	**Gedunin –** 35.95 µg/ml	1281	58.8	41.1
4	**Gedunin –** 35.95 µg/ml	1214	62.4	37.6



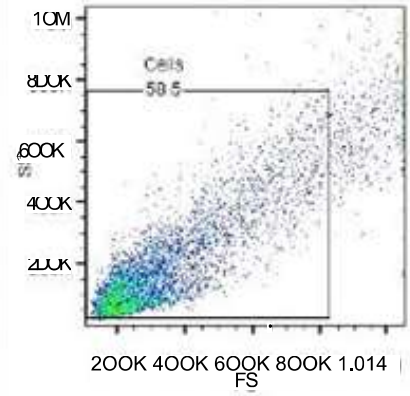


O_Caspase3 HepG2 02Unlrealed 00007049 730 LLD ungatea
10000

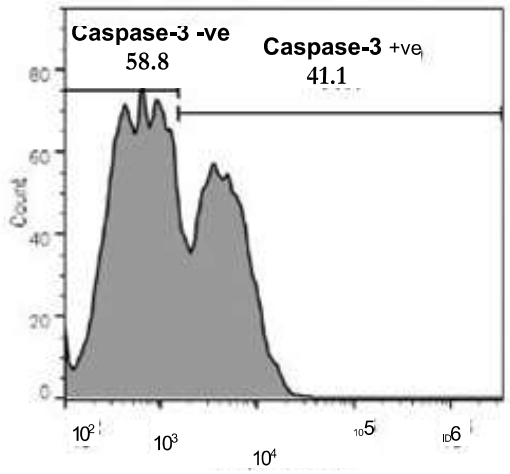


Q_Caspase3 HepG2 O2Untreated 00001049 730.1.1.0 Cells6O93

Cespase.3 +ve: Freq. of Parent : 7.34
Caspase-3 -ve: Freq. Of Parent **92.6 Cells:**
Geometric Mean : *FZI: 490*



Caspasc3 HepG2 O3Gcdunin 00001050 731.1.1AD Ungaled
10000



C-³sOase³ nept32 o3Geounin 00001050 731
LMD celaso46

Caspase-3 **+ve: Freq. of Parent** :41.1
Caspase-3 Ate: **Freq. of Parent : 58.8 Cells:**
Geometric Mean : *FL):* **1281**

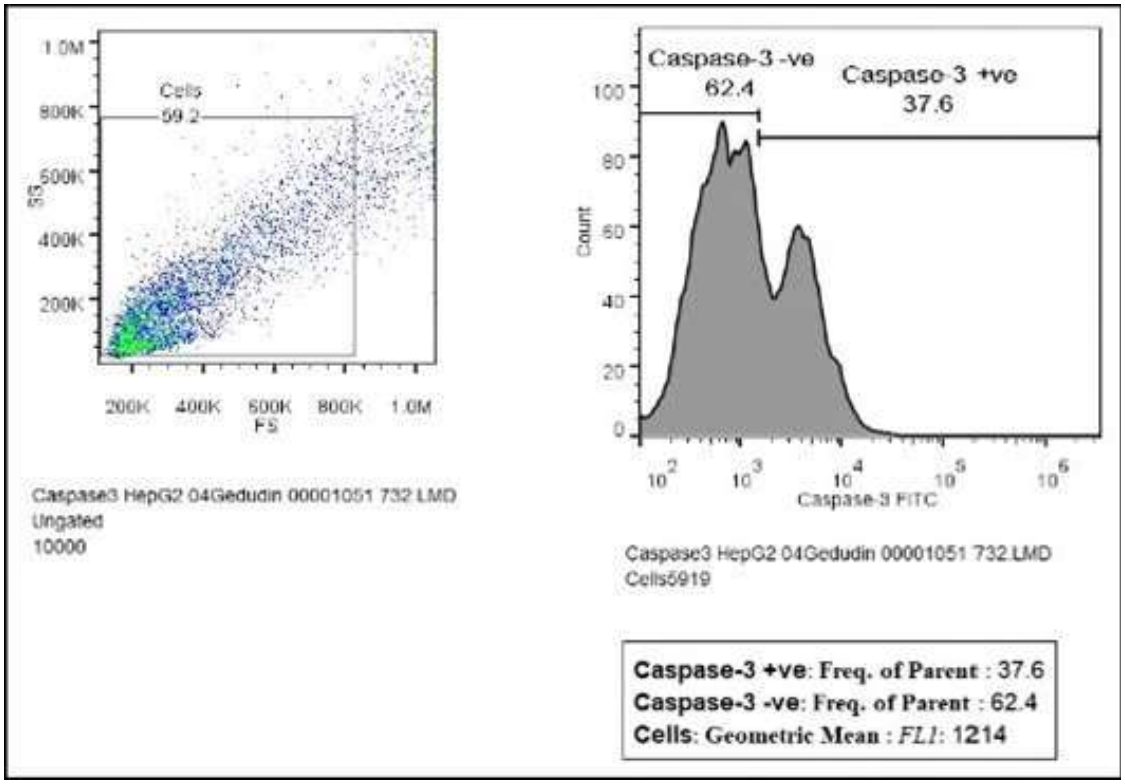


Figure 9.17. Caspase -3 / Apoptosis of HepG2 cell line using flow cytometry.

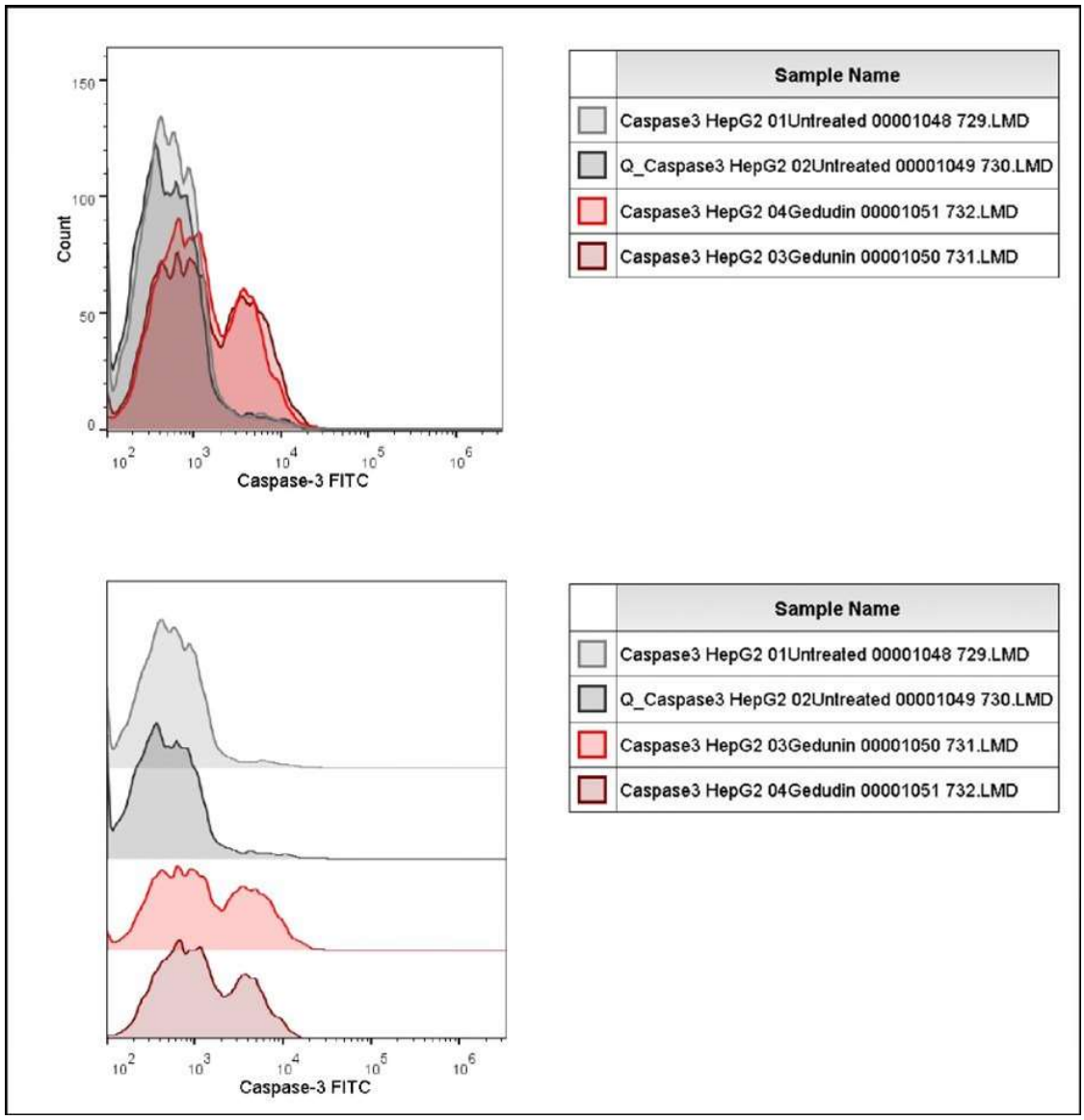


Figure 9.18. Apoptosis / caspase-3 assay of HepG2 cell line.

9.3.6. Evaluation of cell migration/scratch assay using HepG2 cell line, PA1 and PC3 cell line and non-cancerous cell line (NIH/3T3).

The scratch-wound test is a simple, repeatable method for measuring fundamental cell migration properties such as speed, persistence, and polarity. Cells are grown to confluence before introducing a narrow "wound" with a pipette tip. At the wound edge, cells polarise and move into the wound space **(Dai *et al.*, 2017).** The

test investigates the movement of a two-dimensional sheet of cells in response to the creation of a crude wound **(Table 9.5, 9.6 and 9.7).** Cells can either break loose from the sheet or maintain an uninterrupted front. When a scratch is introduced, migration begins from a standstill in a well-defined path - perpendicular to the wound edge. Deviations from this route are easily quantified, yielding directional persistence and speed data in wild-type and modified cells **(Figure 9.19, 9.20, 9.21 and 9.22).**

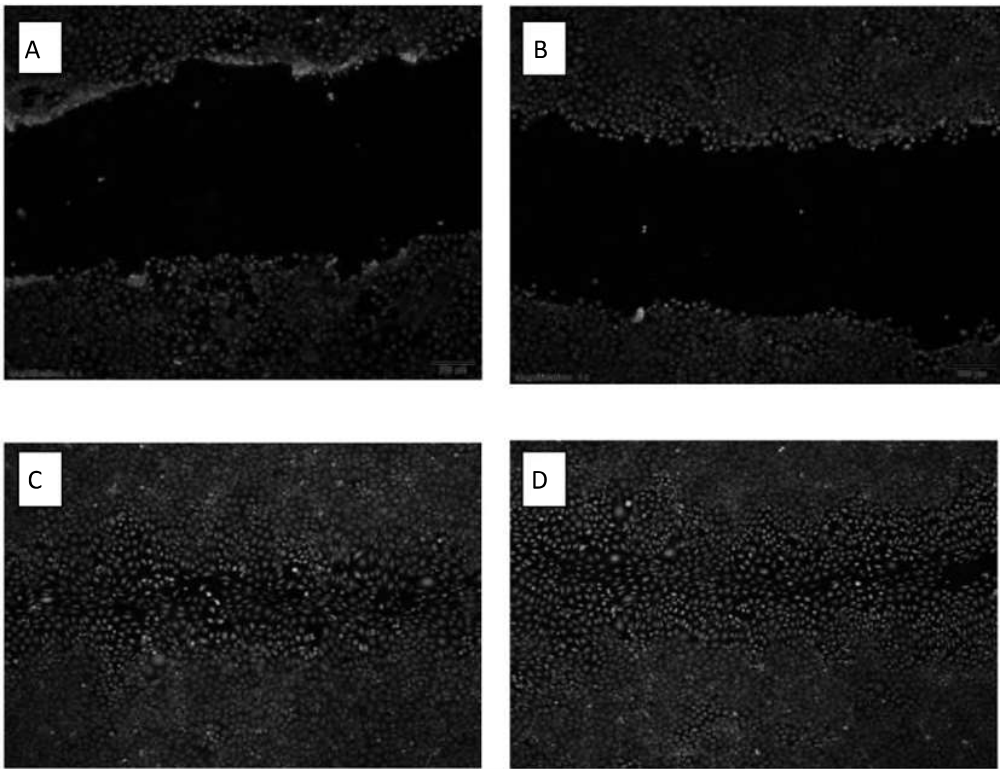


Figure 9.19. Scratch assay of NIN/3T3 cell line showing cell migration in the presence of gedunin from 0 hour to 24 hours.

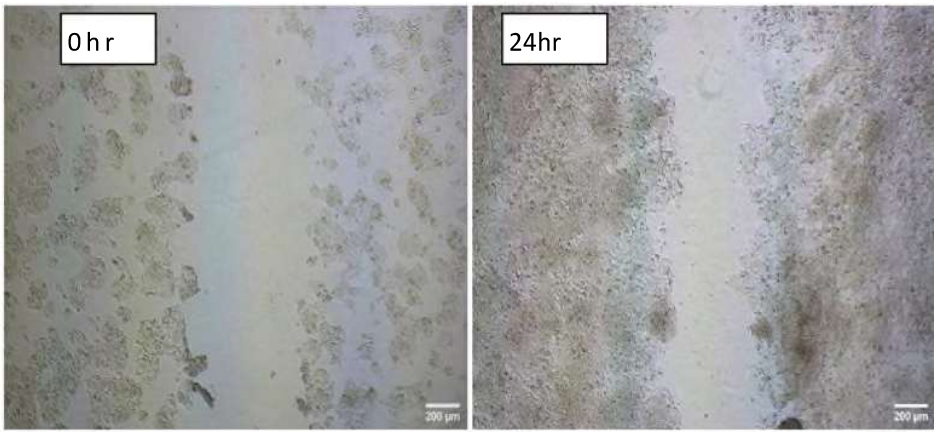


Figure 9.20. Scratch assay of HepG2 cell line showing cell migration in presence of gedunin from 0 hour to 24 hours.

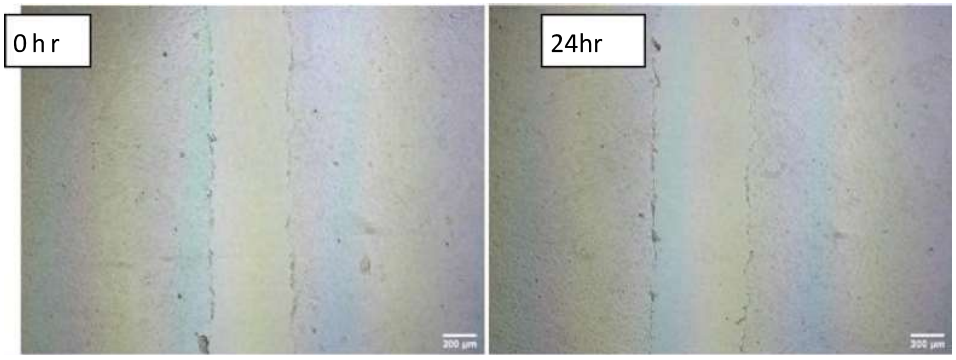


Figure 9.21. Scratch assay PA1 cell line showing cell migration in the presence of gedunin from 0 hour

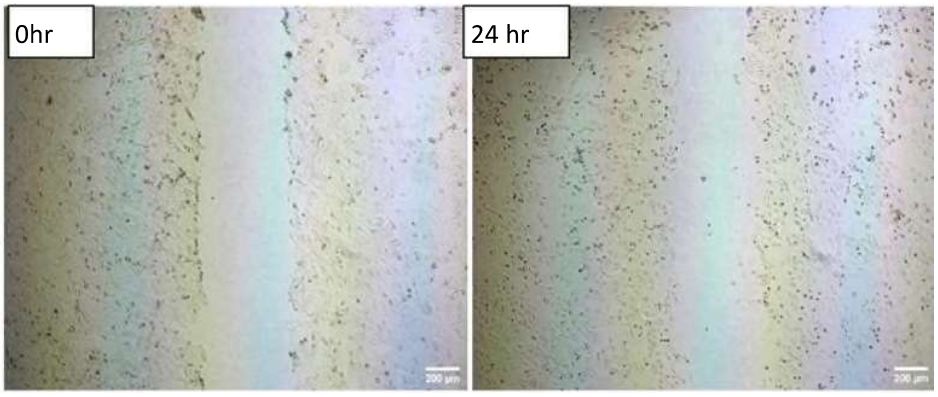


Figure 9.22. Scratch assay PC3 cell line showing cell migration in presence of gedunin from 0 hour to 24 hours.

Table 9.5. Scratch assay of HepG2 cell line.

Cell Migration/Scratch assay - HepG2 cell line								
Sample			Scratch area		% migration	Average (% migration)	% Standard deviation	% Standard error
			0h (i	24h)				
Control	Well 1	Reading	137238	770352	43.87	46.21	11.56	5.78
		Reading 1	125665 7	527713	58.01			
	Well 2	Reading 2	123717 2	596487	51.79			
		Reading 1	122535 4	843071	31.20			
Gedunin - 18µg/ml	Well 1	Reading 2	11659 12	124784	-7.03	-1.32	8.20	4.10
		Reading 1	123310 6	11106 37	9.93			
	Well 2	Reading 2	12591 09	135724 2	-7.79			
		Reading 1	123460 7	123962 2	-0.41			
Gedunin - 36µg/ml	Well 1	Reading 2	130526 6	133497 0	-2.28	2.46	3.35	1.67
		Reading 1	141584 3	137975 3	2.55			
	Well 2	Reading 2	14401 59	136472 3	5.24			
		Reading 1	131692 5	126015 5	4.31			
Sample			Scratch area (in sq. µm)		% migration	Average (% migration)	% Standard deviation	% Standard error
			0h	48h				
Control	Well 1	Reading	137238	133653	90.26	95.95	4.09	2.04
		Reading 1	125665 7	42841	96.59			
	Well 2	Reading 2	123717 2	37830	96.94			
		Reading 1	122535 4	0	100.00			
Gedunin - 18µg/ml	Well 1	Reading 2	11659 12	108750	6.72	0.96	10.03	5.01
		Reading 1	123310 6	138427 9	-12.26			
	Well 2	Reading 2	12591 09	127232 5	-1.05			
		Reading 1	123460 7	110578 1	10.43			
Gedunin - 36µg/ml	Well 1	Reading 2	130526 6	131072 7	-0.42	3.22	7.67	3.84
		Reading 1	141584 3	136837 1	3.35			
	Well 2	Reading 2	14401 59	12409 37	13.83			
		Reading 1	13169 25	13683 19	-3.90			

Table 9.6. Scratch assay of PA1 cell line.

Cell Migration/Scratch assay - PA-1 cell line

Sample			Scratch area (in sq. µm)		% migration	Average (% migration)	% Standard deviation	% Standard error
			0h	12h				
Control	Well 1	Reading 1	872061	262603	69.89	76.04	7.83	3.91
		Reading 2	964432	207052	78.53			
	Well 2	Reading 1	972977	135917	86.03			
		Reading 2	959817	290661	69.72			
Gedunin - 37µg/ml	Well 1	Reading 1	1029667	665313	35.39	34.76	3.07	1.54
		Reading 2	987190	632231	35.96			
	Well 2	Reading 1	1030418	645361	37.37			
		Reading 2	1004773	700086	30.32			
Gedunin - 74µg/ml	Well 1	Reading 1	934058	749288	19.78	12.24	6.20	3.10
		Reading 2	919545	846320	7.96			
	Well 2	Reading 1	966304	904081	6.44			
		Reading 2	1090317	929376	14.76			

Sample			Scratch area (in sq. 0h	Scratch area (in sq. 24h	% migration	Average (% migration)	% Standard deviation	% Standard error
Control	Well 1	Reading 1	872061	0	100.00	100.00	0.00	0.00
		Reading 2	964432	0	100.00			
	Well 2	Reading 1	972977	0	100.00			
		Reading 2	959817	0	100.00			
Gedunin - 37µg/ml	Well 1	Reading 1	1029667	631149	38.70	42.61	11.29	5.65
		Reading 2	987190	515742	47.76			
	Well 2	Reading 1	1030418	463513	55.02			
		Reading 2	1004773	713958	28.94			
Gedunin - 74µg/ml	Well 1	Reading 1	934058	700634	24.99	15.26	13.06	6.53
		Reading 2	919545	923104	-0.39			
	Well 2	Reading 1	966304	875289	9.42			
		Reading 2	1090317	795850	27.01			

Table 9.7. Scratch assay of PC3 cell line.

Cell Migration/Scratch assay - PC3 cell line

Sample			Scratch area (in sq. µm)		% migration	Average (% migration)	% Standard deviation	% Standard error
			0h	12h				
Control	Well 1	Reading 1	1002743	352250	64.87	58.69	7.73	3.87
		Reading 2	1005393	383519	61.85			
	Well 2	Reading 1	1094534	430847	60.64			
		Reading 2	1045592	549951	47.40			
Gedunin - 48µg/ml	Well 1	Reading 1	1052257	909218	13.59	11.68	2.05	1.03
		Reading 2	1037689	903330	12.95			
	Well 2	Reading 1	1109063	1009008	9.02			
		Reading 2	1125965	1000444	11.15			
Gedunin - 96µg/ml	Well 1	Reading 1	1084766	983567	9.33	6.94	11.6	5.85
		Reading 2	1060791	904009	14.78			
	Well 2	Reading 1	1089931	938517	13.89			
		Reading 2	1064847	1173740	-10.23			

Sample			Scratch area (in sq. µm)		% migration	Average (% migration)	% Standard deviation	% Standard error
			0h	24h				
Control	Well 1	Reading 1	1002743	40479	95.96	92.63	5.62	2.81
		Reading 2	1005393	53207	94.71			
	Well 2	Reading 1	1094534	48123	95.60			
		Reading 2	1045592	164861	84.23			
Gedunin - 48µg/ml	Well 1	Reading 1	1052257	1011504	3.87	11.40	7.67	3.84
		Reading 2	1037689	939008	9.51			
	Well 2	Reading 1	1109063	863875	22.11			
		Reading 2	1125965	1012317	10.09			
Gedunin - 96µg/ml	Well 1	Reading 1	1084766	1055955	2.66	-0.54	2.85	1.43
		Reading 2	1060791	1056899	0.37			
	Well 2	Reading 1	1089931	1100859	-1.00			
		Reading 2	1064847	1109269	-4.17			

9.3.7. Evaluation of colony formation/cell proliferation assay using HepG2 cell line, PA1 and PC3 cell line.

The clonogenic cell survival assay measures a cell's capacity to proliferate forever and so keep its reproductive capabilities to create a large colony or clone. This cell is thus referred to as clonogenic. A cell survival curve is thus defined as a connection between the dose of the insulting substance and the proportion of cells that preserve their capacity to reproduce. Although clonogenic cell survival tests were first established to research the effects of radiation on cells and have since played an important role in radiobiology, they are now routinely employed to investigate the effects of drugs with potential clinical uses. In addition to ionising radiation, they include chemotherapeutic medicines such as etoposide and cisplatin, as well as antiangiogenic therapies such as endostatin and angiostatin, and cytokines and their receptors, either alone or in combination therapy.

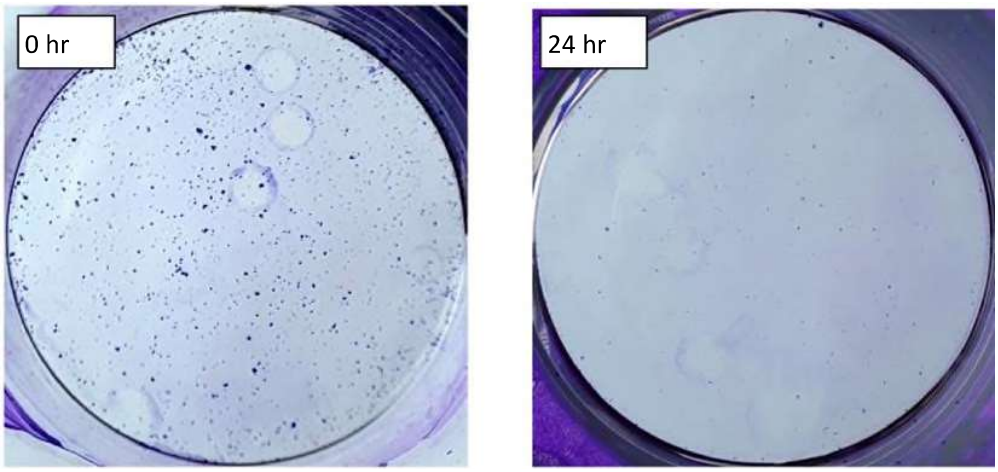


Figure 9.23. Colony formation assay of HepG2 cell line showing cell migration in presence of gedunin from 0 hour to 24 hours.

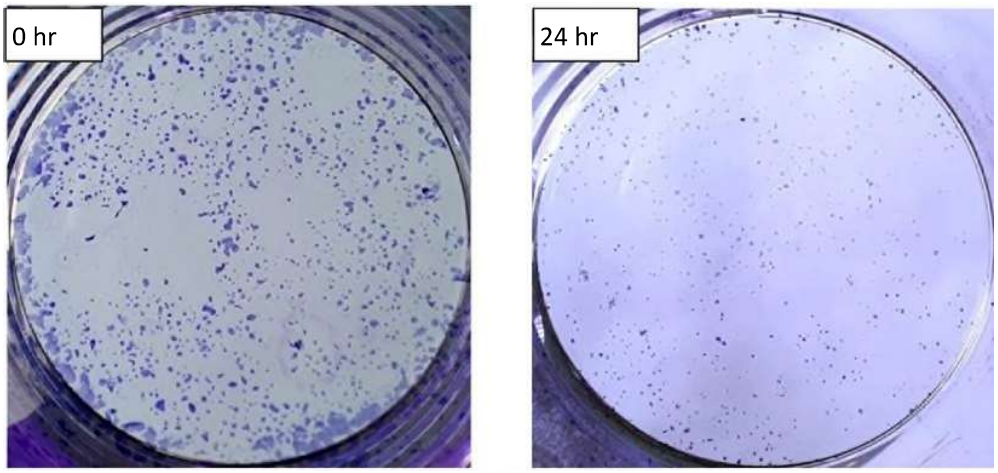


Figure 9.24. Colony formation assay of PA1 cell line showing cell migration in presence of gedunin from 0 hour to 24 hours.

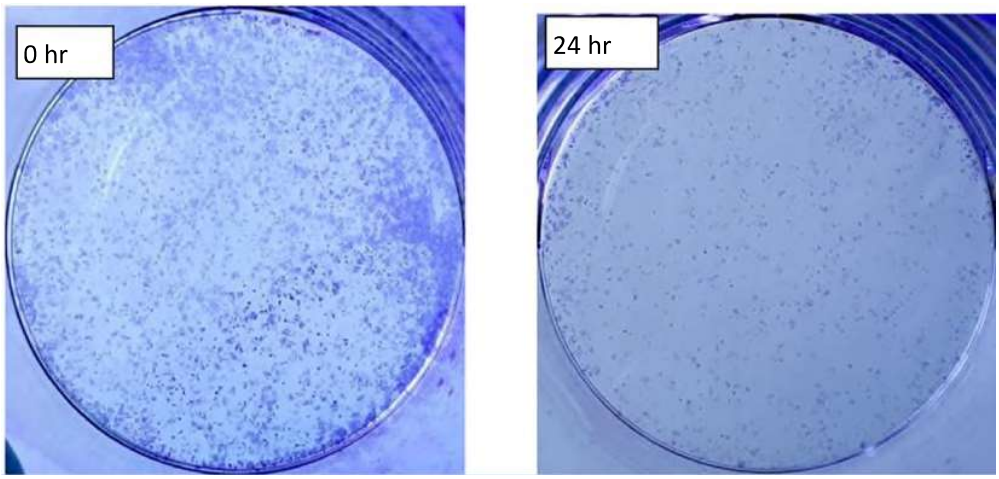


Figure 9.25. Colony formation assay of PC3 cell line showing cell migration in presence of gedunin from 0 hour to 24 hours.

Table 9.8. Colony formation assay of HepG2 cell line

Colony formation/Clonogenic cell survival assay - HepG2 cell line vs Gedunin			
		Gedunin	
	Untreated	**18µg/ml**	**36µg/ml**
No. Of cells plated	500	500	500
Colony count well 1	437	232	26
Colony count well 2	466	271	61
Plating efficiency	90.3	50.3	8.7
Surviving fraction	1	0.557032115	0.096345515

Table 9.9. Colony formation assay of PA1 cell line

Colony formation/Clonogenic cell survival assay - PA-1 cell line vs Gedunin			
		Gedunin	
	Untreated	**37µg/ml**	**74µg/ml**
No. Of cells plated	1000	1000	1000
Colony count well 1	819	636	303
Colony count well 2	954	679	346
Plating efficiency	88.65	65.75	32.45
Surviving fraction	1	0.741680767	0.366046249

Table 9.10. Colony formation assay of PC3 cell line

Colony formation/Clonogenic cell survival assay - PC3 cell line vs Gedunin			
		Gedunin	
	Untreated	**48µg/ml**	**96µg/ml**
No. Of cells plated	1000	1000	1000
Colony count well 1	865	622	284
Colony count well 2	910	633	302
Plating efficiency	88.75	62.75	29.3
Surviving fraction	1	0.707042254	0.330140845

9.3. Conclusion

In silico analyses of the alpha-fetoprotein (AFP), marker revealed that the optimum position had the lowest energy score of -9.38. In the MTT experiment, the $_{IC50}$ value of gedunin was 35.95 g/ml, but gedunin plus $_{H2O2}$ therapy resulted in high ROS levels of 34.4 in liver cancer cells. Gedunin also enhanced active Caspase-3 expression in HepG2 liver cancer cells by 41.1 fold. In silico analyses using CEA receptors present in both PA1 and PC3 cell lines revealed the lowest binding energy at the active site of -9.15 and -7.96 kcal/mol, respectively. In vitro investigations have revealed that gedunin inhibits the growth of PA1 and PC3 cells. Caspase activity was also shown to be 57.4 and 47.9 fold higher in PA1 and PC3 cell lines, respectively.

Scratch assay or migration assay of NIN/3T3 (non-cancer cell line) cell line showed complete cell migration in presence of gedunin from 0 hour to 24 hours, whereas in HepG2 cell line, 46.21 % cell migration was seen in control and 2.46

% cell migration after 24 hours of gedunin treatment (36 µg/ml gedunin/74.6Mm gedunin). In PA1, 100 % cell migration was seen in control and 15.26 % cell migration in 74 µg/ml or 153.34 mM gedunin-treated cells after 24 hours. Furthermore, in PC3 92.63 % cell migration was seen in control and -0.54% in 96 µg/ml gedunin-treated cells after 24 hours. In colony formation assay/cell proliferation as say the control showed 1 surviving fraction in the case of HepG2, PA1 and PC3 cell lines whereas 0.96, 0.36 and 0.33 surviving fraction after 24 hours in the case of HepG2, PA1 and PC3, respectively. Hence gedunin has the potential to act as an anti-cancer agent.