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# Biogenic Synthesis of Cadmium Sulfide Nanoparticles Using Daruharidra (*Berberis aristata*) and Its Implementation as a Novel Therapeutic Agent against Human Breast and Ovarian Cancer

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## ABSTRACT

In this article we have illustrated a novel biogenic and green approach for the production of CdS Nanoparticles (CdS NPs) within 3 nm-5 nm range using an excellent ayurvedic herb known as Daruharidra (*Berberis aristata*). Natural and chemical free daruharidra powder was utilized as a stabilizing agent in the green or biogenic synthesis of CdS based nano formulation. The foremost objective of this study was to explore prospects of these nanoparticles as a potent anticancer agent. Human ovarian teratocarcinoma cells (PA1) and human breast cancer cells (MDAMB-231) were tested against these nanoparticles. Analysis by MTT assay showed cytotoxic effect on cell lines when treated with increasing concentration of NPs. $IC_{50}$ for 24 hour treatment was obtained as 97.34 µg/ml and 809.75 µg/ml for ovarian and breast cancer cells respectively. Further the flow cytometry analysis were performed to understand the apoptosis and cell cycle arrest mechanism. It was found that treatment with CdS NPs lead to significant rise in early apoptotic cells *i.e.* from 2.53% to 13.5% and 3.67% to 12.6% in PA1 and MDAMB-231 cell line respectively. Also the NPs initiate the cell cycle arrest in $G_2$/M phase with sub$G_1$ DNA damage in PA1 cells and $G_0$/$G_1$ arrest in MDAMB-231 cells. The NPs were also tested for their potential as bactericidal agent, which showed excellent results against both gram positive and negative bacteria.

**Keywords**: Cadmium sulfide; Nanoparticles; PA1; MDAMB-231; Anticancer; Herbal medicine

## INTRODUCTION

All over the world the major leading cause of death is cancer; in 2012, there were approximately 14 M new cases of the disease, which is estimated to be 8.2 M deaths worldwide. The united states alone spent 147 billion $ on cancer care in 2017, which is likely to increase because of an exponential increase in new cases with each passing year. Ancient ayurvedic samhitas have mentioned some treatment strategies for cancer. Traditional herbs used to target cancer in various parts of the body have always been in our scriptures. Major cancer targeting drugs present in the market are mostly derived from herbs and plants.

Herbs play a paramount role in chemoprevention and are recognized as the origin of naturally occurring antioxidants. They prevent cells from lipid peroxidation and diseases resulting from it, one of them being cancer. A known syndrome is observed during cancer, which

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is significantly managed by herbal remedies. This syndrome is a cancer cachexia syndrome that involves metabolic changes and immune responses due to any tumor growth in the body. The syndrome includes anorexia, chronic nausea, and other changes in body image. Herbs used in cancer treatment result in reducing the side effects of the syndrome and cancer-associated complications without the use of any other extra medication. It was found that herbal diterpenoid Pseudolaric Acid B (PAB) was potent against the more toxic compound triptolide in the apoptosis and growth of cancerous colon cells. The herbal extract of the brucea and psoralea plant help in treating carcinomas of the cervix, rectum, liver, colon, leukoderma, and much more. Brucea extract, when applied topically on tumors, has the potential to kill tumor cells. Psoralen causes unspecific membrane damage by binding to DNA and causing apoptosis. In the medicaments of various diseases, including cancer, daruharidra comprises many compounds explored as leads. In the past also, many plant-acquired products have been used for anticancer therapy, such as taxol, vinblastine, vincristine, teniposide, etc. For any plant with anti-carcinogenic abilities, it could be mere serendipity or some very high level of ethno-pharmacological screening. In the fields of biochemistry, for the discovery of leads in cancer treatment, Daruharidra extracts in recent years are being explored. Chromatography techniques enable researchers to find hits in any plant extracts that could be used as an anti-carcinogen or anti-tumorigenic agent in many cell lines. The two main active compounds specific to daruharidra or *Berberis* species that can be utilized as leads for developing numerous drugs are berberine and berbamine. Daruharidra is an ayurvedic plant from the scriptures which should not be forgotten; instead, it should be brought back to the present medicine industry as a potent plant that can solve many mysteries of untreatable diseases. In the contemporary world, many young scientists are focusing their work on herbs and plants that can decipher the knot of cancer from the world. Studies suggest using daruharidra in various cancerous cell lines and have shown positive results. This article focuses on *Berberis* sp, thus summarizing various researchers' valuable work on the effects of its extracts and active phytochemicals against cancer. There is innumerable evidence of the protective effects of herbs and plant species working effectively again several types of cancer. The cytotoxic effects of anticancer medication used nowadays affect the receiver and the front-line doctors; this article discusses the analytical methods that can be used as a natural remedy in cancer treatment. *Berberis* sp.'s potential to target cancer has been discussed that relates to the underlying mechanism of its action. Berberidaceae family consists of many antioxidants, alkaloids, and other phytoceuticals that have shown incredible results in the apoptosis of cells. It helps reduce free radicals and treat other complications occurring during cancer. *Berberis* sp. extracts are believed to have shown immunoprotective effects *in vitro* that helps in the prevention of damage due to drugs by its high antioxidant abilities. This study encourages researchers to build a perspective for the withering of life threatening aspects of cancer using natural remedies. The plant carries chemical constituents like berberine, berbamine, oxyberberine, aromoline, palmatine, oxyacanthine, karachine, and taxilamine which helps in targeting cancer in their unique ways. Berbamine, a critical constituent, was found to have $IC_{50}$ values as low as 1.67 mg/mL, which reduced liver cancer by up to 70%. Berbamine belongs to the bisbenzylisoquinoline group of alkaloids. Many plants belonging to the *Berberis* genus and reported to contain berbamine are utilized in traditional Chinese medicine and ayurveda.

Cadmium Sulfide (CdS) belongs to the semiconductor nanoparticles' II-VI group. Due to quantum captivity, these nanoparticles have one of kind optoelectronic properties that significantly differ from the bulk material. These nanoparticles having low-dimensional semiconductor properties are receiving significant attention in cancer treatment. Targeted drug delivery is major concern in cancer nanotechnology. Nano-composed therapeutics is now budding candidates to treat several diseases. Biological treatment imposes precise and targeted drug delivery and shut out side effects originating from the systemic distribution of chemicals from cytotoxic drugs it is found to effectively control cancer cell proliferation and/or tumor angiogenesis. In a standard practice CdS NPs are made from chemical synthesis which is a tricky and cost intensive process requiring synthetic toxic chemicals and several down streaming steps. Therefore, out of ordinary approaches are required in this area to allow synthesis utilizing non-hazardous materials while maintaining the nano size, physicochemical properties, lower cytotoxicity, and biocompatibility. In relation to this, nanoparticles derived *via* biogenic synthesis using biosurfactants (microbes and plants) are a promising approach. Recent research on CdS NPs derived from biological stabilizing agents reveal exceptional quantum confinement effect having elevated photo emission, and eminent antibacterial activity. The chief upper hand of the biogenic synthesis method is that non-toxic compounds from plant and microbial extracts are used as a stabilizing agent to govern the particle range of the CdS NPs. It is much effective in a way as it does not incite any major damage to the cells.

In this article we discuss an off-center, swift and green approach to synthesizing cadmium sulfide nanoparticles using the environmentally safe plant of daruharidra. This method is advantageous as it makes the downstream processing easier, the nanoparticles can be extracted efficiently. It also helps to unearth the latest approach to targeted delivery of anticancer therapeutics. Nanoparticles obtained from herbal sources show promising targeted drug delivery and anticancer properties with low toxicity. CdS NPs are well known for their optical activity and are found to be useful in various applications. They can be used precisely for bio-imaging and drug delivery in cancer treatment. Further, bio inspired CdS NPs as an anticancer drug delivery system is less explored. Thus, this featured article aims to conclude the recent advances in bioinspired CdS NPs for drug delivery in cancer therapy [1-8].

## MATERIALS AND METHODS

Chemicals cadmium sulfate 99.99% and sodium sulfide 98% were obtained from SRL. Water used in the experiment was obtained from the MiliQ water purification system. Cell line-PA1 human ovarian cancer cell line, MDAMB231 human breast cancer cell line, NCCS Pune. Dulbecco's Modified Eagle Medium with High Glucose (DMEM-HG) supplemented with 10% Foetal Bovine Serum (FBS) used for cell culture, MP biomedicals, Germany 1 X Dulbecco's Phosphate Buffered Saline (DPBS), 0.25% Trypsin-EDTA solution, MTT reagent, were all purchased from MP biomedicals, Germany. Dimethyl Sulfoxide (DMSO), cell culture grade, Merck, Germany. PI/RNase staining solution, BD biosciences (Catalog no. 550825). Annexin V- AbFlourTM 488 apoptosis detection kit (KTA0002), Abbkine, Inc.

### Preparation of Extract

The plant (stem and roots) of daruharidra were procured from a local herb shop (Varanasi, India). For sterilization surface of the plant, its parts were washed under tap water several times and then with distilled water. They were then were subjected to drying under shade. The dried plant was ground to powder form, and 10 gm of this was mixed with 100 ml of methanol. This setup was kept for 24 hour incubation at 180 rpm with 40°C. Then the macerated solution of plant was passed through the whitman number 1 filter paper. The methanolic extract was stored at 4°C for further use.

### Green Synthesis of CdS Nanoparticles

The process of preparation of CdS nanoparticles was mainly divided into 2 steps. For the first step 1 mL of 0.025 M $CdSO_4$ was added to 15 mL of daruharidra extract and kept for 72 hours of incubation in the dark. In the second step, 0.25 mL of 0.025 M $Na_2S$ was added and incubated for 96 hours to produce CdS nanoparticles. After the incubation, a bright green color change was observed that indicates the formation of nanoparticles. The solution was then centrifuged at 10000 rpm for 10 min twice. The contamination in the recovered CdS nanoparticles was removed by washing the powder thrice with miliQ water. The pellet was lyophilized for further characterization studies.

### Characterization of CdS Nanoparticles

FTIR spectra analysis was performed using the FTIR imaging system provided by SAIF (IIT Bombay). 3000 hyperion microscope with vertex 80 FTIR system model of bruker, Germany. Infrared spectroscopy gives information on the vibrational and rotational modes of motion of a molecule, and hence it is imperative for identifying the functional groups present on the organic sample. Both the crude extract and lyophilized nanoparticles were subjected to FTIR micro-ATR imaging to analyze the changes in the sample after the formation of Cd nanoparticles.

The lyophilized sample of nanoparticles was subjected to DLS (Dynamic Light Scattering) by Malvern zeta sizer-nano-s to the size distribution of the particles. Nanoparticles were dispersed in methanol and kept the working temperature set to room temperature. The study the surface changes and analysis of elements of the CdS powdered sample was noted by FEG-SEM (JEOL JSM-7600 F). It was coupled with SAXS-xenocs SAS model xeuss 2.0, *i.e.*, small-angle x-ray scattering studies, an analytical characterization tool used to determine the structure of particle systems in terms of averaged sizes, clustering and shapes. The precise shape, aggregation and crystalline nature of the resultant CdS lyophilized powder sample were studied using high resolution transmission electron microscopy HRTEM; JEOL JEM 2100 F).

### Antibacterial Effects of CdS NPs

The antibacterial activity of cadmium sulfide nanoparticles was studied by a well-diffusion method against four bacterial species namely *E. coli*, *S. marcescens*, *S. aureus*, and *B. subtilis*. The cultures were inoculated in Luria Bertani (LB) broth and incubated for 24 hours in a incubator at temperature 37°C and 180 rpm. The thus grown bacterial culture (100 µl) was spread on freshly prepared LB agar petri dishes with a sterile glass spreader and was left to dry for few minutes. Then 6 mm diameter wells were cut on agar plates to hold the test compound. Two different concentrations of CdS nanoparticles (10 µg/mL and 20 µg/mL) were loaded onto the wells and plates incubated for 24 hours. After incubation, the clear zone of antibacterial activity was measured with a millimeter ruler.

### Anticancer Potential of Nanoparticles

**Cytotoxic activity of CdS nanoparticles by MTT assay:** PA1 and MDAMB-231 cells were cultured in T-25 flasks. They were then trypsinized and aspirated into a 5 mL centrifuge tube. Cell pellet was obtained by centrifugation at 300x g. The cell count was adjusted, using DMEM-HG medium, such that 200 µl of suspension contained approximately 10,000 cells. To each well of the 96 well microtiter plate, 200 µl of the cell suspension was added and the plate was incubated at 37°C and 5% $CO_2$ atmosphere for 24 h. After 24 h, the spent medium was aspirated. 200 µl of different test concentrations of test drugs were added to the respective wells. The plate was then incubated at 37°C and 5% $CO_2$ atmosphere for 24 h. The plate was removed from the incubator and the drug containing media was aspirated. 200 µl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5 mg/mL and the plate was incubated at 37°C and 5% $CO_2$ atmosphere for 3 h. The culture medium was removed completely without disturbing the crystals formed. Then 100 µl of solubilization solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank, and concentration of test drug needed to inhibit cell growth by 50% ($IC_{50}$) was generated from the dose-response curve for the cell line.

### Cell Cycle Arrest Analysis

Both PA1 and MDAMB-231 cells were cultured in a 6 well plate having density of 3 × $10^5$ cells per 2 ml and were then incubated in a $CO_2$ incubator at 37°C for 24 hours. The spent medium was aspirated and cells were washed with 1 ml 1 X PBS. The cells were treated with $IC_{50}$ concentration of biogenic CdS nanoparticles in 2 ml of culture medium and then incubated for 24 hours. Negative control was taken with cells left untreated. Chilled 70% ethanol was added drop by drop to cells kept on ice while mixing for fixation. This was done to ensure minimum clumping of cells as ethanol fixed cells require higher centrifugal speeds to pellet compared to unfixed cells. The supernatant was discarded carefully so as to not to lose the pellet. The pellet was subjected to washing twice with PBS. 400 µl PI-RNase solution per million cells was administered and mixed. Cells were incubated for 15 minutes at room temperature. Samples were analysed by flow cytometry in PI/RNaseA solution.

### Apoptosis Assessment

This study was carried on by taking cells in a 6 well plate having density of 3 × $10^5$ cells per 2 ml and then they were incubated in a $CO_2$ incubator at 37°C for 24 hours or overnight. The spent medium was then aspirated and was subjected to washing with 1 ml PBS. The culture cell was treated with CdS NPs $IC_{50}$ concentration in 2 ml of culture medium and was incubated for approx 20 hours. One of the wells was left untreated which was to be used as negative control. After the incubation of cells with nanoparticles, medium was removed from each well and into 5 ml centrifuge tubes and washed with 500 µl PBS. PBS was removed and 200 µl of trypsin-EDTA solution was added and further the culture cells were incubated at 37°C for 5 minutes. The culture media was poured back into their respective wells and the cells were harvested directly into the centrifuge tubes. Centrifugation was done at 300 x g at 25°C for 5 minutes. Supernatant was discarded. Washing was done twice with PBS and then it was decanted completely. Cells were resuspended in 1 X Binding Buffer at a concentration of 1 × $10^5$ cells/ml, transferred 100 µl of the solution to a 5 ml culture tube. AbFlour 488 Annexin V -5 µl was added to the tubes. After vortexing the cells they were incubated for 15 min at room temperature in the dark. 2 µl of PI and 400 µl of 1 X binding buffer to each tube was added and mixed gently. Results were analyzed by flow cytometry immediately after addition of PI [9-16].

## RESULTS AND DISCUSSION

### Structural Analysis

FTIR informs about the functional group analysis of crude methanolic extract of daruharidra had 600-650 halogen compound, 1500 nitrogen compound, 1360 sulfur containing compound, 3300-4400 primary aliphatic amines, 1700-CO unsaturated ester. 2000-1600 aromatic compounds. 1250-1350 amines and other aromatic ester are found, which illustrates the existence of alkaloid content in abundance as can be seen in Figure 1.

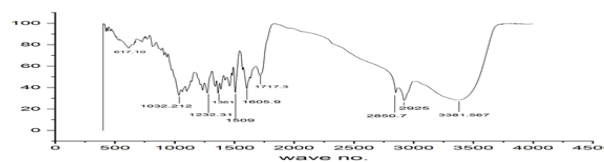


Figure 1: FTIR spectra showing changes in peaks crude plant methanol extract.

This methanolic extract was then used for the production of CdS nanoparticles which showed changes in the FTIR peaks (Figure 2) affirming the generation of the same. 3406.81 shows presence of aromatic primary amino N-H stretch, 2919.19, 2850.38 peaks point to methyl C-H asymmetric stretch, 1590.24 demarcates the carboxylic acid. Specific to attachment of cadmium sulfide bond stretching a peak was observed at 562.08.

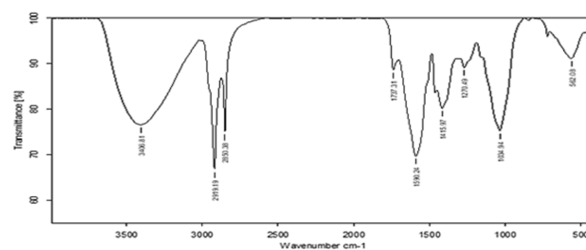


Figure 2: FTIR spectra showing changes in peaks CdS NPs synthesized from it.

In the first attempt to determine the size of the CdS nanoparticles malvern zetasizer nano's was used. The nanoparticles clustering are noticeable apparently due to the high concentration of nanoparticles loaded in the cuvette. The DLS in Figure 3 shows size distribution intensity peak at diameter of 113 nm which could be hydrodynamic intensity due to aggregation of the particles which is further observed in the TEM images.

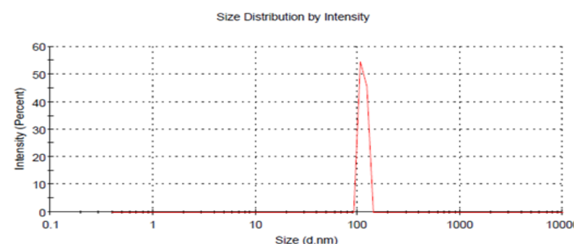


Figure 3: Dynamic light scattering for Cds NPs showing an intensity peak at 113 nm.

FEGSEM images of the same sample depicts spherical or dot like morphology of the CdS nanoparticles. In a similar work presence of oxygen from biogenic CdS was noted when using *Chlamydomonas reinhardii* as a stabilizing agent. Crystal structure properties and particle range of the CdS nanoparticles were studied by HRTEM. It demonstrated that the nanoparticles are uniformly distributed and appear to be clustered. The range of CdS particles lie in 5 nm to 10 nm range. HRTEM indicated single crystalline structure of average

particle size in 3 to 5 nm range, and therefore these particles could also be reviewed as quantum dots (QDs)<10 nm. In several articles, different QD size ranges have been reported (from 4 nm to 10 nm). The average size of CdS QDs is about 10 nm as known from SAXS curves because in the higher Q region (Q>0.07 A-1) where the scattering is dominated by form factor, the SAXS curves can be modeled using a sphere form factor of radius 25 nm. CdS size obtained from the SAXS is the crystal size, which is the smallest and corresponds to the fact that we have polycrystalline or agglomerated CdS. Structural analysis as seen in Figure 4.

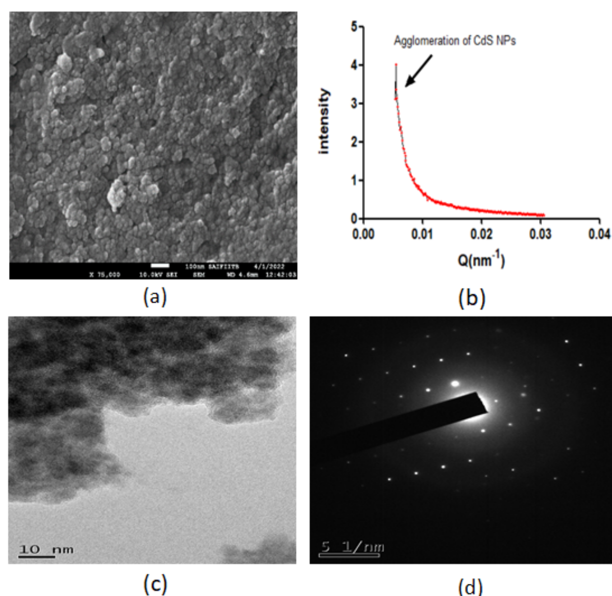


Figure 4: Surface characterization (a) FEGSEM image of CdS NPs; (b) SAXS spectrum; (c) HRTEM image at 10 nm scale; (d) TEM diffraction image scale 5 nm-1 nm.

## Antibacterial Activity

CdS NPs were investigated for their bactericidal properties against gram-positive (*B. sub ls* and *S. aureus*) and gram negative (*E. coli*, *S. marcescens*) bacteria. Any clear area around the wells known as zone of inhibition was observed for both gram positive and gram negative bacteria. At higher concentration of CdS NPs (20 mg/mL) a clear zone with 27 mm diameter is observed for *S. aureus*. In the case of *E. coli* zone of inhibition was measured to 20 mm diameter, respectively as seen in Figure 5. These noteworthy results commend that the CdS NPs performed as a favorable antibacterial agent against both gram positive and negative bacteria. It can be inferred that due to the nano dimension of these CdS particles it was easier for them to penetrate the cell wall of these bacteria and cause plausible damage.

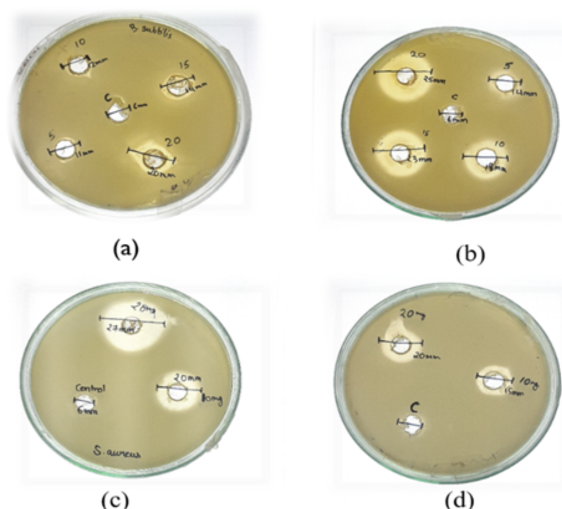


Figure 5: Antibacterial effect of CdS NPs against (a) *B. subtilis*; (b) *E. coli;* (c) *S. aureus*; (d) *S. masercens*. DMSO was taken as control in separate wells.

**Cytotoxic activity of CdS nanoparticles by MTT assay:** Human ovarian cancer cell line PA1 and human breast cancer cell line MDAMB-231 were treated with resulting CdS nanoparticles. Different concentration of NPs was tested on both cell lines using the MTT assay. Viability of cells were observed and calculated over 24 hour timeline. $IC_{50}$ for CdS NP were noted as 97.34 µg/ml and 809.75 µg/ml respectively for PA1 and MDAMB-231 cell line as mentioned in Figure 6. Biogenic CdS particles probably interacted with the phosphorus moieties in DNA. This may have led to inactivation of DNA replication further inhibiting enzyme functions, resulting in loss of viability.

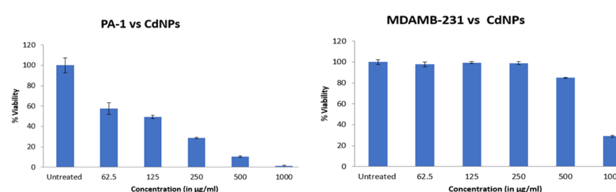


Figure 6: Cytotoxic effect of CdS NPs on PA1 and MDAMB-231 cells tested for 24 hours.

## Apoptosis Assessment

In Figure 7 we observed cell death occurring because of treatment of cancerous cells with CdS NPs; a significant increase in AbFlour 488 fluorescence was observed in the cells treated with CdS NPs in comparison to the untreated control. Increase in the number of early apoptotic cells from 2.53% to 13.5% in PA1 cell line and 3.67% to 12.6% in MDAMB-231 cell line was observed. Increase in the late apoptotic/necrotic population in PA1 was from 1.67% to 5.62% and 1.09% to 19.58% in MDAMB-231 cell line. Cells with increase in only presidiums fluorescence were considered to be dead cells. The Annexin V AbFlour 488 fluorescence was collected in the FL1 detector using a 525 nm

band pass filter and propidium iodide fluorescence was collected in the FL3 detector using a 620 nm band pass filter.

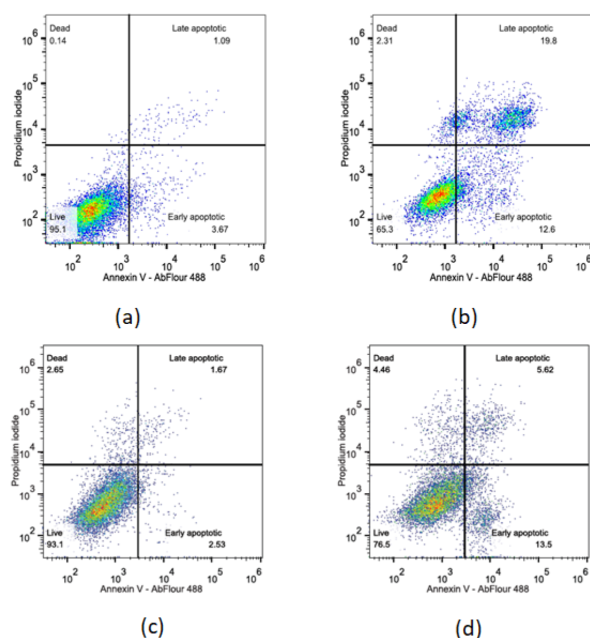


**Figure 7:** Apoptotic cell death analysis by FACS in MDAMB-231 (a) Untreated; (b) Cells treated with CdS NPs and PA1; (c) Untreated; (d) Cells treated with CdS NPs. Apoptotic cells are seen in top and bottom right quadrant showing early and late apoptosis.

### Cell Cycle Analysis by Flow Cytometry

FACS or Fluorescence activated cell sorting was employed to calculate the cells/DNA count in different stages of the cell cycle such as $G_0/G_1/S$ and $G_2/M$. Propidium iodide fluorescence was collected in the FL3 detector using a 620 nm band pass filter. As observed in **Figure 8** PA1 cell line when treated with $IC_{50}$ concentration of CdS NPs showed significant increase in the cells in $G_2/M$ phase of cell cycle with decrease of cells in $G_0/G_1$ phase in comparison to the untreated control which suggests an arrest in $G_2/M$ stages of the cell cycle. It was also noted that an increase in cells with DNA damage in subg1 cells was present. For $G_0/G_1$ phase, the cell tally in untreated cells was of 40.6% and gradually plummeted to 28.4% under CdS NPs treatment. Increase in the cells of MDAMB-231 in $G_0/G_1$ phase of cell cycle from 61.7% to 66.0% with diminishing cells in $G_2/M$ phase from 16.7% to 11.7% was observed in the nanoparticle treated sample as compared to the control sample; which suggests an arrest in $G_0/G_1$ stages of the cell cycle.

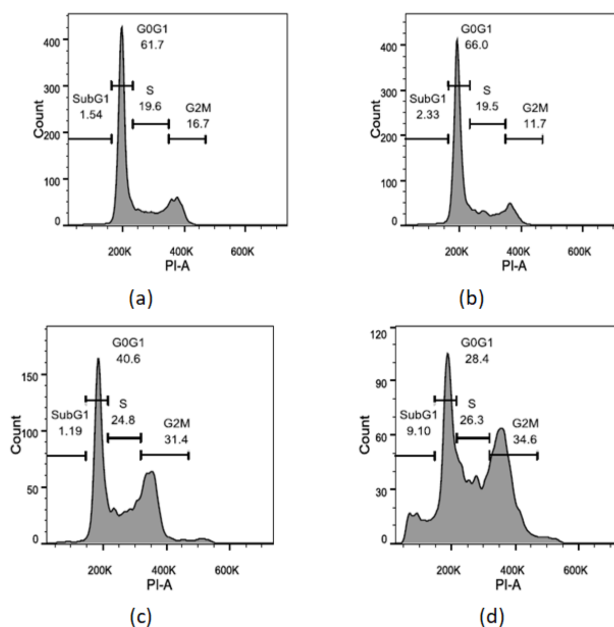


**Figure 8**: Cell cycle arrest analyzed by flow cytometry (a) Control (untreated) MDAMB-231 and CdS NPs at; (b) For MDAMB-23; (c) Untreated PA1; (d) CdS NPs treated PA1 cells.

## CONCLUSION

As the world is progressing there are many drugs that are getting available for targeting cancer. Huge amount of work and energy is getting involved to find an appropriate and aggressive drug that could cure cancer or maybe just weaken its jeopardy to human race. These common cancer drugs are so aggressive in nature that they cause innumerable appalling side effects. It's because of these spinoffs scientists are looking into ways that could target cancer and have minimal negative effects on patients. *Berberis* sp extract can be an intelligible alternative that bears positive effects on people suffering from this disease. The availability of more specific herbal medicine should be more useful to prevent or treat cancer. *Berberis* has various benefits other than treating cancer. The positive effects of the extract on cells of the human body can help the recovery of a patient as a whole. It is an unconventional approach. Thus, researchers are encouraged *via* this review to conduct more research in this field and give credible verification suggesting the use of *Berberis* plant extract in oncology [17-23].

## CONFLICT OF INTEREST

We wish to draw the attention of the editor to the following facts that there are no potential conflicts of interest and to significant financial contributions to this work.

## FUNDING

NA

## ETHICAL COMPLIANCE

This article does not contain any studies involving human or animal subjects performed by any of the authors.

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