Original Article

Induced Apoptotic Potential of Green Synthesized AgNPs from Sargassum Wightii on Human Prostate Cancer (PC-3) Cells

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Abstract

The present study was aimed to explore the apoptotic potential of marine brown algae Sargassum wightii and it's AgNPs against human prostate cancer (PC-3) cells in vitro. The AgNPs of S.wightii caused inhibition in cell viability than that of aqueous extract. AgNPs, caused high intense positive staining of nucleus of PC-3 cells than that of S. wightii aqueous extract, thus clearly depicting that AgNPs induced cell death even at minimum concentration than that of aqueous extract.DNA damage in AgNPs treated PC-3 cells was high than that of aqueous extract when assessed by Comet assay, which was well depicted by head and tail DNA content in it.DNA laddering assay revealed that cells treated with aqueous extract and AgNPs showed DNA fragmentation. This study clearly demonstrates that AgNPs are effective in inducing DNA fragmentation when compared to aqueous extract. Collectively, the data confirmed that AgNPs had induced cell death through apoptotic pathway. Aqueous extract of S.wightii gated 59.75% of PC-3 cells in sub-G0/G1 phase, while AgNPs gated 78.97% of PC-3 cells in sub-G0/G1 region. The significant reduction of the DNA content made them appear as an indication of apoptosis, with consequent loss of cells in the G1 phase. Interestingly, cell cycle results revealed that AgNPs arrested all stages of the cell cycle and found to cause more damage to the DNA than that of aqueous extract. The study in toto confirmed that S.wightii and its AgNPs have profound apoptotic potential.

Keywords: Sargassum wightii, AgNPs, Annexin V-FITC and PI, Comet, DNA fragment, Cell cycle.

Introduction

Nano-medicine is an emerging field expanding rapidly because of the development and incorporation of new nano composites into a range of products and technologies.[1] In recent years, the application of nanoparticles (NPs) in medicine has increased and expanded to the fields of molecular imaging,[2] drug delivery,[3] diagnosis and treatment of cardiovascular diseases,[4] wound healing,[5] antioxidant,[6] anticancer[7] and development of materials and medical devices with antimicrobial properties.[8] New applications of nanoparticles and nanomaterials are emerging rapidly in biomedical sciences.[9,6]

Prostate cancer is the second most common cause of cancer and the sixth leading causes of cancer death among men worldwide. It has become a major health problem in industrialized world during the last decades of the 20th century contributing to three fourth of the registered cases across the globe.[10] A number of new strategies are being developed to control and treat cancer. Of which, combination of photochemical with chemotherapeutic identified as an effective approach in reducing the toxicity to normal tissues. Since 1950's onwards the plant derived metabolites are served as potential anti-cancer drugs.[11]

Sargassum wightiiis one of the important species belonging to the genus Sargassum and a wide range of bioactive properties have been reported from this species.[12] It is widely distributed on the southern coasts of Tamil Nadu, India, and many parts of Asia and it is reported to be used as animal feed, food ingredients and fertilizer. Since Sargassum wightiiis available in large quantities, it appears to be the most suitable raw materials for commercial exploitation. Sargassum wightiialso shows a good amount of flavonoids in support and its antioxidant activity[13] indicating that this genus is an ideal target for investigating the activity of the biomolecules present in Sargassum wightiifor various medical and industrial applications.[14]

Work on synthesis of nanoparticle using algae as a source has been unexplored and unraveled and

recently there are only very few reports regarding the use of algae as a bio-factory for synthesis of metallic nanoparticles. Moreover, no work has been reported on the anticancer activities of AgNPs from Sargassum wightii(Greville) by any researchers and interestingly, we were the first to report on the green synthesis of AgNPs from Sargassum wightiand assess its antioxidant potential and anticancer activity against PC-3 prostate cancer cells in vitro[12] and the in silico molecular docking of alginic acid and fucoidan compound present in Sargassum wightii against apoptotic proteins (Caspase-3, Caspase-9 and β-Actin).[15] In continuation of that the anti-apoptotic potential of Sargassum wightiiand its AgNPsagainst prostate cancer (PC-3) cell line in vitro has been explored in the present study.

Materials and methods

Sargassum wightii was collected from Central Salts and Marine Chemicals Research Institute-Marine Algal Research Station, (CSMCRI-MARS), Mandapam Coast, Tamil Nadu. The sample was identified as Sargassum wightii (Phaeophyta) by Dr. K. Krishnamoorthy of Krishnamoorthy Institute of Algalogy, Chennai, Tamil Nadu, India. After collecting, the seaweed was washed well with running tap water was shade dried for ten days and the dried seaweed was crushed to fine powderusing kitchen blender. Then, 20 g of dried fine powderwas taken and mixed with 200 ml of the respective solvents (methanol, chloroform, ethyl acetate, hexane and distilled water) and kept in a boiling water bath at 60°C for 10 min. The extract was filteredwith Whatman filter paper No. 1. The filtered extract was stored inrefrigerator at 4°C until further use.[12]

Biological synthesis of AgNPsfrom Sargassum wightiiwas carried out in the five different polar solvent extracts and characterized as outlined.[12] Synthesis of AgNPs was best in aqueous extract only and hence aquesous extract and AgNPs of Sargassum wightiiwere taken for further studies.

Cell Viability

Human prostate cancer (PC-3) cell line used for the present study was proccured from National Centre for Cell Science (NCCS), Pune, India. Cell viability of PC-3 cells was assessed with aqueous and AgNPs of Sargassum wightiiby MTT method (Mosmann, 1983).The 50% inhibition of cell viability was recorded at 109.88 µg/ml and 49.48 µg/ml for aqueous extract and AgNPs, respectively for 24 h. As the incubation period was increased to 48 h, the aqueous extract and AgNPs of Sargassumwightii gave an IC_{50} value of 40.59 µg/ml and 8.84 µg/ml, respectively as reported in our previous study.[12] Further studies were carried out with IC_{50} 48 h concentrations of aqueous extract and AgNPs of Sargassumwightii.

Nuclear staining with annexin V-FITC and propidium iodide

Apoptosis and nuclear morphology in the control and treated PC-3 cells was analyzed using apoptosis detection kit (Sigma St. Louis, USA) according to the manufacturer's protocol.[16] Briefly, PC-3 cells ($1x10^6$/well) were plated in six-well plates and allowed to attach overnight, and then treated with an IC_{50} 48 h concentration of aqueous extract and synthesized AgNPs and control cells were maintained separately for 48 h. Cells were harvested and washed twice with PBS. The cells were re-suspended in 500 µl of 1X binding buffer, stained with 5.0 µl of annexin V-FITC conjugate and 10.0 µl of propidium iodide solution and incubated for 15 min in the dark at room temperature. Stained cells were analyzed to be viable (annexin V-FITC and PI negative), early apoptotic (annexin V-FITC positive and PI negative) or late apoptotic (annexin V-FITC and PI positive) by FAC scan flow cytometer (Becton Dickinson). The degree of apoptosis was quantified as a percentage of the annexin V-FITC and PI positive cells. The nuclear morphology results were confirmed by viewing the cells under a fluorescence microscope, using filters appropriate for Annexin V-FITC/PI stain. Nuclear morphology of cells was examined under Carl Zeiss Axio vision fluorescent microscope (Software: Axiovision 4.8).

Determination of DNA damage by comet assay

DNA damage in the cell suspension was done.[17] The PC-3 cells were treated with 48 h IC_{50} concentration of aqueous extract and AgNPs separately for 48 h. The control cells received only 0.1% DMSO. The treated cells were collected and DNA was isolated, and the isolated DNA was used for agarose electrophoresis. For this, the cells were washed with PBS and then 0.5 ml of lysis buffer was added, transferred to a microfuge tube and incubated for 1 h at 37°C. To this, 4.0 µl of proteinase K was added and the tubes were incubated at 50°C for 3 h. To each tube, 0.5 ml of phenol: chloroform: isoamyl alcohol (25:24:1) was added, mixed well and centrifuged at 10,000 rpm for 10 min. to separate the DNA containing upper aqueous phase. Phenol-chloroform-isoamyl alchohol extraction was repeated twice, followed by chloroform extraction alone. To the resulting aqueous phase, 2 volumes of ice-cold absolute ethanol and 1/10th volume of 3.0 M sodium acetate were added and incubated for 30 min. on ice to precipitate DNA. The precipitated DNA was pelleted by centrifuging at 13,000 rpm for 10 min. at 4°C, the supernatant was aspirated and the pellet was washed with 1.0 ml of 70% ethanol. After repeating the above centrifugation step and removing the last traces of the supernatant fraction, the pellet was allowed to dry at room temperature for approximately 30 min. and it was re-suspended in 50 µl of TE buffer and the DNA was quantified by UV-visible spectroscopy.

Exactly 5.0 ml of 1% normal agarose was dissolved in phosphate buffer saline at 65°C and dropped on to fully frosted slides and covered immediately using a cover slip and placed over a frozen ice pack for about 5 min. The cover slips were removed after the gel had solidified. One fraction of the treated cell suspension (IC_{50} 48 h concentration of aqueous extract and AgNPs) was mixed with three fractions of 1% of low melting point agarose at 37°C. About 100 µl of the prepared cell suspension-agarose mixture was quickly dropped on to the gel and coated over the micro-slides and was allowed to solidify. A third coating of 100 µl of l% low melting agarose was given on the gel containing the cell suspension and was allowed to solidify. Similarly, the slides were prepared for each cell fraction. The gel coating was allowed to polymerize and the cover slips were removed and immersed in ice-cold lysis solution at 4°C for 16 h. All the above steps were performed under low lighting conditions to avoid any additional damage.

Electrophoresis was performed using a horizontal electrophoresis system. The prepared slides were gently removed from the lysis solution and placed horizontally in an electrophoresis tank. The electrophoresis buffer was filled into the tank until the slides are just immersed in the buffer. The slides were allowed to stand in the buffer for about 20 min. (to allow DNA unwinding) and electrophoresis was performed at 0.8 Volts/cm for 15 min. The power supply was disconnected and the slides were carefully removed and rinsed thrice in neutralization buffer and gently dabbed dry. A few drops of working solution of ethidium bromide were added onto the gel and covered using a cover slip. The stained DNA in the cells was examined at 200 X and 400 X magnifications using fluorescent microscope equipped with a 365 nm excitation filter and a 435 nm barrier filter. Finally, the lengths of migrated DNA (Comet tail) were measured using the CASP software and about 10-50 comets/points were scored.

DNA fragmentation study

DNA fragmentation study was carried out by DNA extraction and agarose gel electrophoresis.18Cancer cells (3×105 cells/ml) were plated per well in 6 well plates with DMEM medium containing 10% FBS. The cells were incubated for 12 h under 5% CO_2, 95% O_2 at 37°C. Then, the RPMI-1640 with FBS was removed and washed with PBS. The medium was removed and washed with PBS and then fresh medium was added and kept for 1 h in the incubator. Then, the medium was removed and the control wells received again medium and treatment plates received 40.59 µg/ml and 8.84 µg/ml i.e., 48 h IC_{50} concentrations of aqueous extract and AgNPs containing medium. Then, the culture plates were again incubated as above.

After completion of incubation time, the DNA was extracted from the cell lysate. For this, the cells were washed with PBS and then 0.5 ml of lysis buffer was added, transferred to a microfuge tube and incubated for 1 h at 37°C. To this, 4.0 µl of proteinase K was added and the tubes were incubated at 50°C for 3 h. To each tube, 0.5 ml of phenol: chloroform: isoamyl alcohol (25:24:1) was added, mixed well and centrifuged at 10,000 rpm for 10 min. to separate the DNA containing upper aqueous phase. Phenol-chloroform-isoamyl alcohol extraction was repeated twice, followed by chloroform extraction alone.

To the resulting aqueous phase, 2 volumes of ice-cold absolute ethanol and 1/10th volume of sodium acetate were added and incubated for 30 min. on ice to precipitate DNA. The precipitated DNA was pelleted by centrifuging at 13,000 rpm for 10 min. at 4°C, the supernatant was aspirated and the pellet was washed with 1.0 ml of 70% ethanol. After repeating the above centrifugation step and removing the last traces of the supernatant fraction, the pellet was allowed to dry at room temperature for approximately 30 min. and it was re-suspended in 50 µl of TE buffer. The DNA was quantified by UV-Visible spectroscopy and 10.0 µg of DNA was electrophoresed in 1% agarose gel containing ethidium bromide in a mini gel tank containing TBE buffer for 1 h under 90 V. Then, the gel was examined under UV transilluminator (BioRad) and photographed.

Cell cycle analysis (flow cytometry)

The cell cycle phase's distribution and measurement were assessed by flow cytometry.[19] PC-3 cells were incubated in serum free DMEM for 36 h to synchronize cells in G0/G1 Phase. Then, the medium was replaced by 10% serum-supplemented medium with 0.1% DMSO (as control) and 40.59 µg/ml (aqueous extract IC_{50} 48 h concentration) and 8.84 µg/ml (AgNPs IC_{50} 48 h concentration). After treatment, floating cells in the medium were combined with attached cells and collected by trypsinization. Cells were washed with cold PBS and fixed in 80% ethanol in PBS at -20°C. The fixed cells were pelleted and stained with PI (50 µg/ml) in the presence of RNaseA (20 µg/ml) for 30 min. at 37°C. About ≥ 20,000 cells were analyzed in a Becton Dickinson FAC scan flow cytometer. Cell cycle histograms were analyzed using Cell Quest software.

Results

Nuclear staining with annexin V-FITC and propidium iodide

The cells stained with annexin V-FITC, PI and annexin V-FITC/PI was observed using confocal microscope and the images are presented in Figure. 1. The PC-3 control cancer cells stained negative for annexin

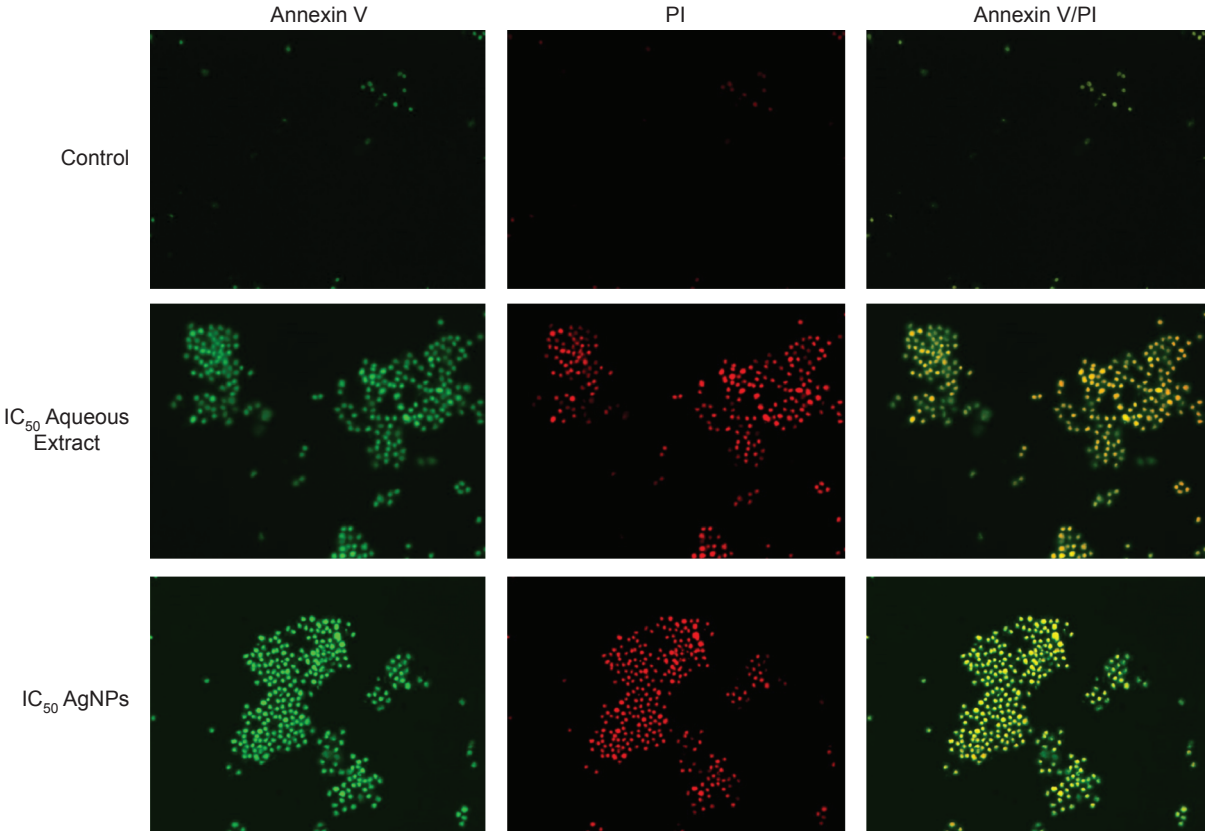

Figure 1: Annexin V-FITC, PI and annexin V-FITC/PI nuclear staining when treated with 48 h IC50 concentration of aqueous extract and AgNPs of Sargassumwightii

V-FITC, PI and annexin V-FITC/PI. In contrary, PC-3 cells treated with IC_{50} 48 h concentration of aqueous extract and AgNPs showed intense positive staining with annexin V-FITC, PI and annexin V-FITC/PI. When compared among aqueous and AgNPs, the latter treatment revealed high intense positive staining that the former treatment. These results clearly depicts that AgNPs induced cell death even at minimum concentration, when compared with that of aqueous extract.

Determination of DNA damage by comet assay

The nuclei of PC-3 cells (control as well as treated) were stained with propidium iodide and visualized under fluorescence microscope and are presented in Figure. 2. Comet assay results indicated that DNA damage occurred in aqueous and AgNP treated cells, while in control cells, DNA damage was not visible. The damage was much higher in AgNP treated cells than that of aqueous extract treated cells. The fragmented DNA migrating out of the nucleus appeared as comet like tail cells. The results of quantitative analysis of DNA damage by using CASP software are presented in Table 1. The results illustrates that more DNA damage was observed in AgNP treated PC-3 cells when compared to aqueous extract treated cells, which is well depicted by head and tail DNA content. In contrary in the control cells, the DNA remained intact without any damage in it.

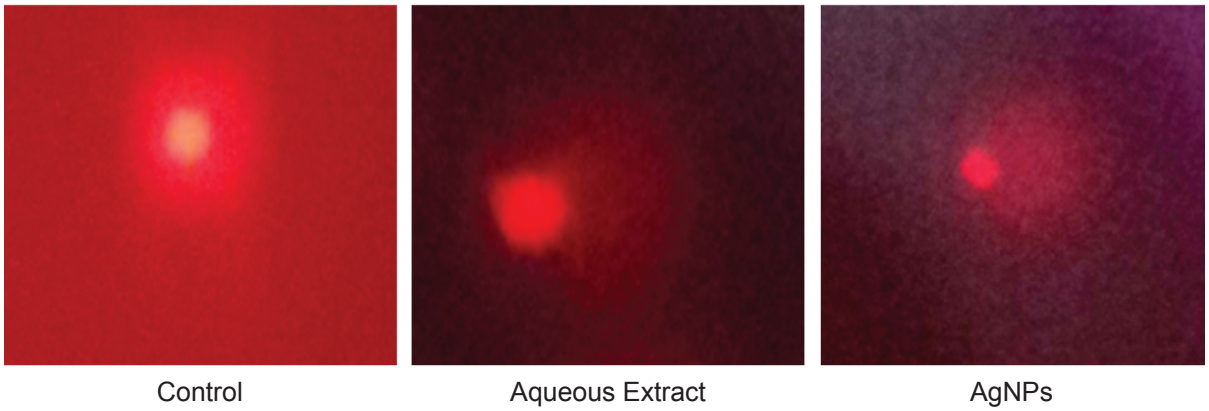

Figure 2: DNA damage and comet formation in PC-3 cells when treated with IC 50 48 h concentration of aqueous extract and AgNPs of Sargassumwightii

Samples	Head	Tail	Comet	Head DNA	Tail DNA	Tail movement	Olive tail movement
Control	125	20	145	95.0544	4.94561	0.989122	2.74403
Aqueous extract	81	70	151	66.7157	33.2843	23.298	19.222
AgNPs	93	74	167	54.4715	43.528	32.2111	20.3384

Table 1: Quanitative DNA fragmentation analysis of PC-3 cells when treated with 48 h IC_{50} concentration of aqueous extract and AgNPs of Sargassum wightiiby CASP software

DNA Fragmentation study

AgNPs induced DNA damage was further confirmed by DNA laddering assay. DNA fragmentation analysis is a typical assay to confirm the drug-induced apoptotic cell death. After treatment with 48 h IC50 concentration of aqueous extract and AgNPs, the PC-3 cells were harvested for DNA fragmentation assay. The results revealed that the control cells had intact form of DNA without any fragmentation whereas, cells treated with aqueous extract and AgNPs showed DNA fragmentation (Figure. 3). This study clearly demonstrates that AgNPs are effective in inducing DNA fragmentation when compared to aqueous extract. Collectively, the data confirms that AgNPs had induced cell death through apoptotic pathway.

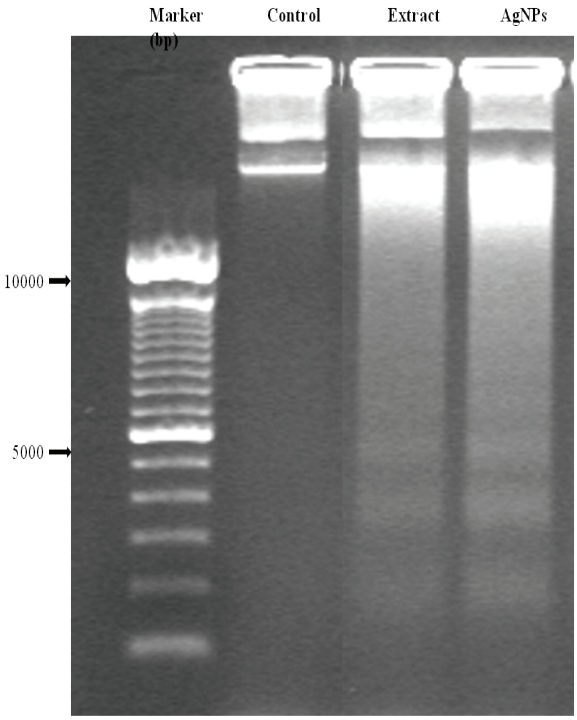

Figure 3: DNA fragmentation ladder assay when treated with 48 h IC50 concentration of aqueous extract and AgNPs of Sargassumwightii

Cell Cycle Analysis

Cell cycle analysis was performed on control PC-3 cancer cells and on PC-3 cells treated with IC_{50} 48 h concentration of aqueous extract and AgNPs (Figure. 4). The graph shows that 34.37% of control PC-3 cells gated in sub-G_0/G_1 phase. On the other

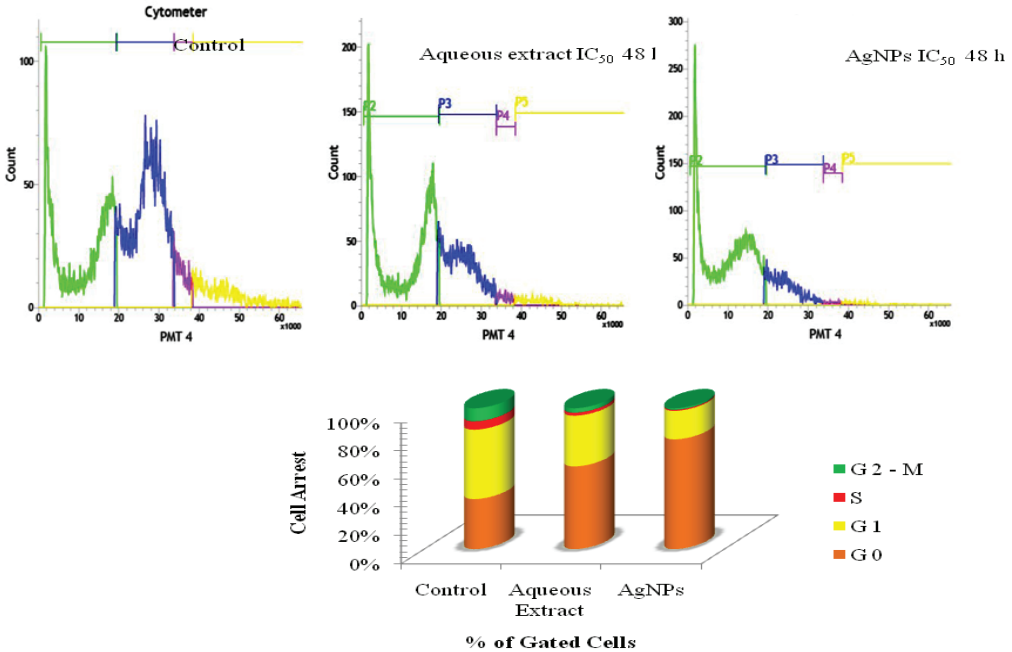

Figure 4: Cell arrest at Go – G1 phase of PC-3 cells induced aqueous extract and AgNPs of Sargassumwightii

hand, 59.75% of PC-3 cells treated with 48 h IC_{50} values of aqueous extract gated in sub-G_0/G_1 phase. Similarly, 78.97% of PC-3 cells treated with 48 h IC_{50} concentration of AgNPs gated in sub-G_0/G_1 region. The significant reduction of the DNA content made them appear as an indication of apoptosis, with consequent loss of cells in the G_1 phase. Interestingly, cell cycle results revealed that AgNPs arrested all stages of the cell cycle and found to cause more damage to the DNA than that of aqueous extract.

Discussion

Thecytotoxic effect of crude extracts from Asparagopsisarmata, Brongniartellabyssoidesand Heterosiphonia plumose on Daudi (Human Burkitt's lymphoma), Jurkat (Human leukemic T-cell lymphoblast), and K562 (Human chronic myelogenous leukemia) cells.[20] Anticancer activity of Sargassum oligocystum aqueous extract against human cancer cell lines (Daudi and K562), which proved that brown alga Sargassum oligocystumhave remarkable antitumour activity.[21] Green synthesis of AgNPs of 10 nm size using Sargassum vulgare and its preferential ability to kill cancerous human myeloblastic leukemic cells (HL60) and cervical cancer cells (HeLa) as compared with normal peripheral blood mononuclear cells.[22] On other hand, work on anticancer potential of green synthesized silver nanoparticles of Sargassum wightii against human prostate cancer (PC-3) cells has not been carried out till date.

In the present study, both aqueous extract and AgNPs significantly reduced the viability of PC-3 cells. The treated cells appeared to shrink, became spherical in shape and cell spreading patterns were restricted. The cells when stained with annexin V-FITC, PI and annexin V-FITC/PI, clearly depicted that aqueous extract and AgNPs induced cell death even at a minimum concentration. Comet assay results indicated that DNA damage occurred in aqueous and AgNP treated cells. The fragmented DNA migrating out of the nucleus appeared as comet like tail cells.Further, quantitative analysis of DNA damage by using CASP software illustrated that more DNA damage was observed in AgNP treated PC-3 cells when compared to aqueous extract treated cells, which is well depicted by head and tail DNA content. AgNPs induced DNA damage was further confirmed by DNA laddering assay. The cells treated with aqueous extract and AgNPs showed DNA fragmentation. Our study clearly demonstrates that AgNPs are effective in inducing DNA fragmentation when compared to aqueous extract. Collectively, the data confirms that AgNPs has induced the cell death through apoptotic pathway.

Our investigation on cell cycle analysis revealed that 34.37% of control PC-3 cells gated in sub-G_0/G_1 phase, 59.75% of aqueous extract treated PC-3 cells gated in sub-G_0/G_1 phase and 78.97% of AgNPs treated PC-3 cells gated in sub-G_0/G_1 region. The significant reduction of the DNA content made them appear as an indication of apoptosis, with consequent loss of cells in the G_1 phase. Interestingly, cell cycle results revealed that AgNPs arrest all stages of the cell cycle and found to cause more damage to the DNA than that of aqueous extract when compared to control. Similar reports were observed on the cytotoxic effect of four species of red algae on Daudi (Human Burkitt's lymphoma), Jurkat (Human leukemic T-cell lymphoblast), and K562 (Human chronic myelogenous leukemia) cells,[20] anticancer activity of Sargassum oligocystum aqueous extract against human cancer cell lines (Daudi and K562)[21] and AgNPs of Sargassum vulgare on cancerous human myeloblastic leukemic cells (HL60) and cervical cancer (HeLa) cells.[22]

Oxidative stress is known to be implicated in the process of carcinogenesis through damage to cellular molecules, such as proteins, lipids and nucleic acids. Hence, the prevalence of both antioxidant and cytotoxic properties in a single compound could be beneficial in terms of rational, preventive or therapeutic purposes. However, the authors further added that additional studies are needed to demonstrate that exhibits no cytotoxicity towards normal cells. Detailed examination of the mechanism of action of the isolated and purified compounds of seaweed extracts should be investigated, whether their impact on cell cycle, their ability to activate caspases or induce mitochondrial and DNA damages.[20]

Apoptosis is a physiological process of killing cells and is an important process to eliminate tumours. The apoptosis process can be characterized by membrane bleeding, shrinkage of cells and nuclear volume, chromatin condensation, DNA fragmentation and formation of membrane bound vesicles which can be triggered by multiple independent pathways by within or outside of the cell.23Green synthesis of AgNPs using Sargassum vulgare and its preferential ability to kill cancerous HL60 and HeLa cells as compared with normal peripheral blood mononuclear cells, DNA fragmentation study and annexin V marker fluorescence-activated cell sorting (FACS) analysis ofAgNP-induced cell death is through apoptosis.[22] The authors' findings revealed the potential utility of AgNPs in the treatment of cancer as prophylactic agent with antioxidant property and chemotherapeutic agent for their selective toxicity to cancer cells.

In the view of understanding the mode of cell death after exposure to NPs, it is recommended that a combination of at least three criteria of cell death to be explored: DNA fragmentation, annexin V binding and/or caspase activation, structural changes in the cell morphology.[24] The cytotoxicity of AgNPs towards cancer cells is due to apoptosis or not, the DNA profiles of the HL60 cells treated with AgNPs along with control cells were analyzed. In control cells, the DNA was not fragmented, whereas the DNA of cells treated with AgNPs was fragmented.[22] A similar DNA fragmentation was seen in aqueous and AgNPs of Sargassumwightii treated PC-3 cells in our study also.

To further evidence the apoptotic activity induced by AgNPs, aliquot of HL60 cells that were incubated with or without the AgNPs and analyzed by flow cytometry (fluorescence-activated cell sorting - FACS). About 87% of the cells treated with the AgNPs underwent apoptosis, whereas only 12% increase in the necrotic cell population of control was observed. A similar arrest of 34.37% of control PC-3 cells gated in sub-G_0/G_1 phase, 59.75% of aqueous extract treated PC-3 cells gated in sub-G_0/G_1 phase and 78.97% of AgNPs treated PC-3 cells gated in sub-G_0/G_1 region were recorded in our study. Cell death induced by AgNPs was through apoptosis. AgNPs started the apoptosis process with fragmentation of DNA. Moreover, cleavage of DNA at the inter-nucleosomal linker sites yielding DNA fragments is regarded as a biochemical hallmark of apoptosis. Flow cytometry measurements for annexin V binding further confirmed the apoptosis-mediated cell death in the cells treated with AgNPs.[22] Their observation was in agreement with previous studies,[25] the ability of AgNPs to induce apoptosis. In contrast, AgNP-induced necrosis in the PC-12 cells.[26] These conflicts will be under debate until a generalised method for the preparation of NP with controlled size, shape and properties is developed. Collectively, the DNA fragmentation and the flow cytometry measurements22 confirmed the apoptotic mode of cell death in cancer cells exposed to AgNPs synthesized by alginate.

Mechanical injury caused by NP depositions in mitochondria might be the reason for mitochondrial damage, which is also expected to have lethal effects in DNA synthesis and DNA damage. Increased alteration and damage to DNA within the cells could cause the change in membrane potential of mitochondria that leads to mitochondrial cytochrome c release, which is a key event in the activation of caspase-3, a downstream pivotal step to initiate apoptosis.[27,22] A similar change in the ultrastructural characteristics such as cell shrinkage, condensation of chromatin, nuclear breakdown, cell membrane leakage and DNA fragmentation were observed in the present study also, when PC-3 cells were treated with AgNPs of Sargassumwightii. There is also a possibility that endocytosis of AgNPs might have occurred into PC-3 cells and got deposited in the mitochondria and nucleus causing that mechanical injury in mitochondria leading to mitochondrial damage, causing lethal effects in DNA synthesis and DNA damage. The increased alteration and damage to DNA within the cells could have caused changes in membrane potential of mitochondria, leading to mitochondrial cytochrome c release, which might be a key event in the activation of caspase-3, a downstream pivotal step to initiate apoptosis.[22,24,25,26,27,28]

Therapeutic index of any material for medicinal applications is very important criterion for the effective therapy of any disease. One of the greatest challenges facing chemotherapy is the inability of anticancer drugs to effectively distinguish between normal and transformed tissues.[29,30,31,32] Interestingly, cancerous HL60 cells were more susceptible to AgNP-mediated toxicity than the normal PBMC with the therapeutic index, i.e., the ratio of toxic dose to effective dose is 22, which is of potential clinical relevance, they concluded that the biosynthesized AgNPs inhibited the lipid peroxidation-mediated reactive oxygen species generation thus preventing the irradiation-related carcinogenesis.[22] The results of the present study also clearly indicates that the antioxidant potential of AgNPs of Sargassumwightii and the endocytosis of AgNPS into the cells might have caused damage to the DNA, thus leading to the process of apoptosis. Owing to its selective toxicity and effective therapeutic index, we ensure that the key findings of the work will create new horizon in the future development in nanotherapy of cancer.

Conclusion

In the present study, the aqueous and extract-based synthesized AgNPsshowed high cytotoxic activity against PC-3 cells to the best of our knowledge. Hence, Sargassum wightiiaqueous extract-based synthesized AgNPscan be used as a potential therapeutic drug due to its bioactive properties. Moreover, identification of active phytoconstituents in the aqueous extracts will pave a way for using this seaweed as a natural antioxidant and cytotoxic agent against various cancers.

References

1. Ashok K, Sivakumari, RajeshS. Achyranthes aspera mediated green synthesis of silver nanoparticles. Indo American Journal of Pharmaceutical Sciences. 5(1), 64-73.

2. Kohl Y, Kaiser C, Bost W, Stracke F, Fournelle M, Wischke C et al. Preparation and biological evaluation of multifunctional PLGA-nanoparticles designed for photoacoustic imaging. Nanomedicine: Nanotechnology, Biology and Medicine. 2011; 7(2), 228-237.

3. Meng H, Liong M, Xia T, Li Z, Ji Z, Zink JI, Nel AE. Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. ACS Nano. 2010;4(8):4539-50.

4. Godin B, Sakamoto JH, Serda RE, Grattoni A, Bouamrani A, Ferrari M. Emerging applications of nanomedicine for the diagnosis and treatment of cardiovascular diseases. Trends Pharmacol Sci. 2010 ;31(5):199-205. doi: 10.1016/j.tips.2010.01.003.

5. Tian J, Wong KK, Ho CM, Lok CN, Yu WY, Che CM, Chiu JF, Tam PK. Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem. 2007; 2(1):129-36.

6. Jayaprakash P, Sivakumari K, Rajesh S, Ashok K. Antioxidant Properties of Green Synthesized Silver Nanoparticles from Sargassum wightii. Saudi J Med Pharm Sci, 2020; 6(8): 516-525

7. Flora J, Sivakumari K, Rajesh S, Ashok K, Jayaprakash P. In vitro antioxidant, anticancer and apoptotic potential of green synthesized silver nanoparticles from propolis against human hepatocellular carcinoma (HepG-2) cells. Alochana Chakra J. 2020; 9(5), 801-820.

8. Rangari VK, Mohammad GM, Jeelani S, Hundley A, Vig K, Singh SR, Pillai S. Synthesis of Ag/CNT hybrid nanoparticles and fabrication of their nylon-6 polymer nanocomposite fibers for antimicrobial applications. Nanotechnology. 2010 ;21(9):095102.

9. Flora J, Sivakumari K, Selvaraj R, Ashok K, Jayaprakash P, Rajesh S. Green synthesis of silver nanoparticles from propolis. Res J Life Sci Bioinform Pharm Chem Sci. 2018; 4, 23-36.

10. Perin NN. Global variation in cancer incidence and mortality. Curr. Sci., 2001; 81: 465-474.

11. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol. 2005 Aug 22;100(1-2):72-9. doi: 10.1016/j.jep.2005.05.011.

12. Jayaprakash P, Sivakumari K, Ashok K, Rajesh S, Prabhu D, Chandrasekar D. Anticancer potential of green synthesized silver nanoparticles of Sargassum wightii against human prostate cancer (PC-3) cell line. Eur. J. Pharm. Med. Res. 2017; 4(3): 275-287.

13. Meenakshi A, ManickaGnanambigai D, Tamil Mozhi S, Arumugam M, Balasubramanian T. Total flavonoid and in vitro antioxidant activity of two seaweeds of Rameshwaram coast. Global J. Pharmacol. 2009; 3(2): 59-62.

14. Iswarya Devi JA, Sathiya Balan G, Periyanayagam K. Pharmacognostical study and phytochemical evaluation of brown seaweed Sargassum wightii. J. Coastal Life Med. 2013; 1(3): 199-204.

15. Jayaprakash P, Sivakumari K, Ashok, Rajesh S. In silico molecular docking of alginic acid and fucoidan compound present in S. wightii against apoptotic proteins (caspase-3, caspase-9 and β -actin). IJBPAS. 2018; 7(8), 1551-1565.

16. Homburg CH, de Haas M, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D. Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. Blood. 1995; 85(2):532-40.

17. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988; 175(1):184-91.

18. Bortner CD, Oldenburg NB, Cidlowski JA. The role of DNA fragmentation in apoptosis. Trends Cell Biol. 1995 ;5(1):21-6. doi: 10.1016/s0962-8924(00)88932-1

19. Pozarowski P, Darzynkiewicz Z. Cell cycle analysis of in vitro cancer cell lines. Curr. Prot. Cell Biol. 2004; 5: 832-836.

20. ZubiaM, Fabre M, Kerjean V, Deslandes E. Antioxidant and cytotoxic activities of some red algae (Rhodophyta) from Brittany coasts (France). Botanica Marina. 2009; 52: 268-277.

21. Zandi K, Ahmadzadeh S, Tajbakhsh S, Rastian Z, Yousefi F, Farshadpour F, Sartavi K. Anticancer activity of Sargassum oligocystum water extract against human cancer cell lines. Eur Rev Med Pharmacol Sci. 2010; 14(8):669-73.

22. Govindaraju K, Krishnamoorthy K, Alsagaby SA, Singaravelu G, Premanathan M. Green synthesis of silver nanoparticles for selective toxicity towards cancer cells. IET Nanobiotechnol. 2015; 9(6):325-30. doi: 10.1049/iet-nbt.2015.0001.

23. Kaufmann SH, Earnshaw WC. Induction of apoptosis by cancer chemotherapy. Exp. Cell Res. 2000; 256: 42–49.

24. Arora S, Jain J, Rajwade JM, Paknikar KM. Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. Toxicol Appl Pharmacol. 2009; 236(3):310-8. doi: 10.1016/j.taap.2009.02.020.

25. Hsin YH, Chen CF, Huang S, Shih TS, Lai PS, Chueh PJ. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicol Lett. 2008; 179(3):130-9. doi: 10.1016/j.toxlet.2008.04.015

26. Kim TH, Kim M, Park HS, Shin US, Gong MS, Kim HW. Size-dependent cellular toxicity of silver nanoparticles. J Biomed Mater Res A. 2012 Apr;100(4):1033-43. doi: 10.1002/jbm.a.34053.

27. Roy S. Caspases at the heart of the apoptotic cell death pathway. Chem. Res. Toxicol. 2000; 13: 961-962.

28. Uren AG, Vaux DL. Molecular and clinical aspects of apoptosis. PharmacolTher. 1996;72(1):37-50.

29. Hellman S. Improving the Therapeutic Index in Breast Cancer Treatment: The Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Research. 2021; 40: 4335-4342

30. Premanathan M, Karthikeyan K, Jeyasubramanian K, Manivannan G. Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. Nanomedicine. 2011;7(2):184-92.

31. Wu YN, Chen DH, Shi XY, Lian CC, Wang TY, Yeh CS, Ratinac KR, Thordarson P, Braet F, Shieh DB. Cancer-cell-specific cytotoxicity of non-oxidized iron elements in iron core-gold shell NPs. Nanomedicine. 2011; 7(4):420-7.

32. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.

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