Original Article

Evaluation of the Cytotoxicity, Apoptosis and Autophagy Induction by Fucoidan in Human Hepatic Adenocarcinoma Cells

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ABSTRACT

Introduction: Seaweeds are the reservoir of bioactive constituents such as proteins, polysaccharides, alkaloids, steroids and terpenoids that possess antioxidant, anti-inflammatory and anti-cancerous properties. *Dictyota bartayresiana* is a brown seaweed with high antioxidant activity, making this species very valuable for human health. Fucoidan is the sulphated polysaccharide obtained from brown algae. **Objectives:** The study aimed to extract, purify and characterize fucoidan from *Dictyota bartayresiana* and investigate its antioxidant and anti-cancer properties. **Materials and Methods:** Fucoidan was isolated from *Dictyota bartayresiana* and was investigated for its antioxidant property by DPPH and ABTS assays, whereas cytotoxicity in SK-Hep-1 cells was checked using MTT assay. The apoptosis assay was achieved via the Annexin-V-Apoptosis detection kit, and images were obtained. To detect autophagy, western blotting was performed for protein Atg5. **Results:** Fucoidan revealed positive radical scavenging actions even at minor concentrations. The results acquired from the MTT assay revealed that cell death initiated at minor concentrations of 0.781μg/ml and was in a dose-dependent mode. The IC_{50} value obtained was 30.96 (μg/ml). In our study, SK-Hep-1 cells revealed that the intensities of the emission bands altered in response to apoptosis. It was observed that after 24 hr of fucoidan treatment, the level of Atg5 increased with an increase in fucoidan concentration and the band size observed for Atg5 was 55kDa. **Conclusion:** Fucoidan possessed antioxidant and cytotoxic properties and was found helpful in managing hepatocellular carcinoma.

Keywords: *Dictyota bartayresiana*, Radical scavenging, Polysaccharide, Antioxidant potential, Apoptosis.

Submission Date: 19-10-2021;
Revision Date: 02-12-2021;
Accepted Date: 16-05-2022.

DOI: 10.5530/ijper.56.3.125

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INTRODUCTION

A large number of cancer deaths occur worldwide due to hepatocellular carcinoma.[1] The Barcelona Clinic Liver Cancer Staging System emphasizes proton therapy for the remedy of hepatocellular carcinoma.[2] Even though surgical resection and liver transplantation have been stated as a cure for hepatocellular carcinoma, the survival rate is lower in patients receiving surgery. Thus, it has become a demanding necessity to explore innovative medications to cure hepatocellular carcinoma.[3] Normal physiological metabolism ensures the continuous release of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide anion.[4] In the case of seaweeds, the liberation of such ROS occurs on the exposure of seaweeds to ultraviolet radiation.[5] ROS provokes peroxidation of lipids and injury of the cell membrane by damaging internal biomolecules, thus leading to cell death. Radical scavengers are thought to be the possible treatment to eliminate all free radicals and their adverse responses.[6] Lower levels of reactive oxygen species lead to numerous diverse effects on cell metabolism, such as cytotoxicity and signal transduction. In contrast, high levels of these reactive oxygen species damage macromolecules, leading to numerous chronic impairments such as carcinomas and hepatic disease.[7] Even an increased

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level of reactive oxygen species may lead to liver fibrosis by causing mitochondrial damage of hepatocytes.[8] An antioxidant is responsible for neutralizing reactive species and radical scavenging, further reducing hepatic injury associated with radicals. Owing to the lack of suitable drugs, natural resources possessing antioxidant properties draws much attention from researchers.[9]

Apoptosis is a programmed cell death comprising two major biological processes: tissue development and conservation of homeostasis.[10] The significant pathways responsible for initiating apoptosis in mammals are the intrinsic pathway and the extrinsic pathway. The Bcl-2 proteins regulate the mitochondria-associated (intrinsic) pathway. The death receptors, together with tumour necrosis factor (TNF) receptor-1, CD95/Fas receptors, govern the death receptor pathway (extrinsic pathway). Therefore, the investigation of innovative drugs targets that trigger apoptosis in tumour cells may lead to novel anti-cancer mediators. Enormous antioxidants are achieved from natural sources, for instance, marine seaweeds, floras, vegetables, as well as fruits.[5] The sulphated polysaccharide is acknowledged to have numerous pharmacological assets, including hepatoprotective actions, antioxidant capability, and anti-inflammatory activities.[11] As a result, our study aimed to extract, purify, and characterize sulphated polysaccharides from *Dictyota bartayresiana* and evaluate cytotoxicity, apoptosis, and autophagy stimulation by fucoidan in SK-Hep-1 human hepatic adenocarcinoma cell lines.

MATERIALS AND METHODS

Extraction of Sulphated Polysaccharides

The sulphated polysaccharide was extracted using a standard protocol. Ten grams of fine particles of sample algae *Dictyota bartayresiana* was incubated the entire night using acetone to eliminate unwanted deposits. The remains were further moistened in 0.25M sodium chloride, and the pH was controlled and brought to 8 using sodium hydroxide. Proteolysis was done by the addition of trypsin and was incubated overnight. Subsequently, the residue was strained with the aid of cheesy fabric, and the remains were separated via frozen acetone with tender blending at 4°C. The residue obtained was centrifuged for 25 min at 10,000 rpm and dehydrated under vacuum. The sulphated polysaccharide obtained was utilized for additional investigations.[12]

Column Chromatography

Column chromatography was used to purify the fucoidan from the sulphated polysaccharide obtained. To purify the sulphated polysaccharide, the crude extract was liquefied by dissolving in 150ml of 0.9% sodium chloride and was fractionated via the DEAE-Sephadex A-50 column that was eluted embracing step gradient of 0.4 M to 2M sodium chloride. The fractions that were positive for carbohydrates were analyzed. The eluents were pooled into several significant fractions.

Carbohydrate and Sulphate Analysis

The carbohydrate content and sulphate content in the total sulphated polysaccharide extract was checked by the standard protocol defined by Dubois *et al.*[13] and Kolmert *et al.*[14] where standards selected were glucose and magnesium sulphate.

Estimation of Total Polysaccharide Content

To estimate the amount of polysaccharides present, a standard curve was developed. A stock glucose solution was prepared containing glucose with a concentration of 1mg/ml. Then different glucose concentrations were used as the standard (0-200 μg) from 1 mg/ml stock solution. Further, the reference solutions were taken in different amounts such as 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1.0 ml of and were put into a 10-ml test tube with a stopper, and volume was made up to 1ml using water. After calibrating the solution volume, 1ml 5 % (w/v) phenol was added, along with 5 ml conc. H_2SO_4 and the tubes were agitated precisely. The tubes were subjected to boiling water bath (approx. 20 min) and were further cooled for another 5 min in an ice bath. A blank was prepared containing only reagents and not glucose. The absorbance was noted at 490 nm, and the graph for the standard was plotted. After the standard curve was achieved, 0.5ml sample solutions were added to the test tubes. Later, a similar procedure as that used for the standard was followed. The polysaccharide amount was determined by employing the absorbance and the standard curve.[15]

Structural Prediction of Sulphated Polysaccharide

Lyophilized form of purified sulphated polysaccharide obtained was subjected to Solid-state-Nuclear magnetic resonance studies for predicting structure. The ^{13}C spectrum of the purified extract of sulphated polysaccharide was investigated.

Identification of Chemical Profile of Sulphated Polysaccharide

The lyophilized purified sulphated polysaccharide was further subjected to Fourier Transform infrared spectroscopic investigation to determine the functional groups present in the polysaccharide obtained. The spectrum obtained was documented.

Radical Scavenging Assay

Radical scavenging action of sulphated polysaccharides was evaluated employing DPPH and ABTS assay against standard control. In the process, numerous concentrations of the sulphated polysaccharide were procured in the test tubes, and the volume was prepared up to 50µL with methanol. In the DPPH assay, 3mL of DPPH solution was added to all the tubes and was incubated in the darkness for 15min. After gestation, the absorbance was noted at 517nm spectrophotometrically with methanol as a blank. Similarly, in the ABTS assay, after the volume in each tube is made up to 50µL with methanol, 3ml of ABTS solution was poured into each tube and was mixed and incubated for 30 min at 20-25°C in a dark area. The sample was further subjected to estimating absorbance at 734nm spectrophotometrically with methanol as a blank.[16] Inhibition percentages were computed as:

$$\text{Inhibition \%} = \frac{\text{Absorbance of Control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Cytotoxic Effect of Fucoidan

SK-Hep-1 cell line gained from National Centre for Cell Science, Pune, India (NCSS) was utilized to investigate the cytotoxicity action through cell viability assay (MTT assay). Minimal Essential Media complemented with 10% FBS, streptomycin (100µg/ml) and penicillin (100 U/ml) in a CO_2 (5%) at 37°C were utilized to preserve cells. Cells were sowed in 96-well plates at 5000 cells/ well and were gestated for 48hr. Numerous concentrations in µg/ml (250, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, and 0.781) of the trial sample were used for 24hr incubation. Afterwards, the medium was removed and was washed using phosphate-buffered saline. The sample to be tested was put in a fresh medium comprising 50µl MTT solution (5mg/ml) to every well and gestated for 4hr. After the gestation period, DMSO was put to dissolve the MTT formazan precipitate formed. The absorbance assessed the viable cells via microplate reader at 570nm. Results obtained were noted as a percentage, and the IC_{50} value was calculated. The percentage of cell viability was estimated via the succeeding formula:

$$\text{Viability \%} = \frac{\text{Absorbance of test samples} - \text{Absorbance of Blank}}{\text{Absorbance of control} - \text{Absorbance of Blank}} \times 100$$

Detection of Apoptosis

Cell apoptosis was examined via Annexin V–FITC/ propidium iodide (PI) staining. In the current analyses, cells were preserved with an altered concentration of treatments and incubated for 24-48 hr, further collected via centrifugation. Further, the cells were rinsed two times using cold PBS. They were suspended again in 1X Binding Buffer. 5 µL of annexin V-FITC and propidium iodide was supplemented and gestated in dark conditions at 20-25°C for 5 min. The detection was done via fluorescence microscopy.

Detection of Autophagy

Western blotting was carried out to examine autophagy, and Atg5 protein is the autophagy-related protein. Therefore, a western blot was performed for Atg5 protein. Culture plates were cleaned with freezing cool PBS. The cells were scraped via a cell scraper, and a Triple detergent lysis buffer was used to lyse cells in the presence of inhibitor phenyl methyl sulfonyl fluoride. Proteins were dissolved in 2X loading buffer and were consequently denatured at 100°C for 10 min. Subsequently, 20-40 µl proteins were loaded onto Tris-glycine gels and electro-transferred onto PVDF (0.2µm, 26.5cm X 3.75 cm). The membranes were gridlocked in 5-10% non-fat dried milk in PBS for 120 min at 20-25°C and nurtured with 1° antibodies in 1% by shaking at 4°C the entire night. After wash, the membranes were nurtured with 2° antibodies at a dilution of 1:2000 at room conditions for an hour. Further DAB was poured into the membrane and incubated at room temperature. Progress was monitored and subjected to filter wash in PBS. Signals were detected using enriched chemiluminescent Western Blotting Detection reagent and captured by the Gel Documentation System.

RESULTS

Extraction and Purification of Sulphated Polysaccharide

Seaweeds serve as a boon for society due to their countless application in the pharmaceutical industry. Several deadly diseases are also cured using marine seaweeds, thus depicting their vital role in every field.[17-18] Thus, the crude extract of sulphated polysaccharide extracted from *Dictyota bartayresiana* was subjected to purification in the present study. The eluents were pooled out of the Sephadex A-50 column as 100 fractions of 1.5ml each. Fractions containing polysaccharides were then identified and fractionated, reliant on their ionic character at several diverse molarities of NaCl, resulting in significant carbohydrate

fractions F1-F5. The fractions were screened for carbohydrates using the phenol sulphuric method and were eluted into five fractions accordingly. Further, these five fractions obtained after the purification by column chromatography were subjected to estimate polysaccharide and sulphate content. It was found that all five fractions yielded a good amount of polysaccharides. In comparison, the first fraction contained the highest amount of polysaccharide, i.e. 1571.89µg/ml, followed by the third fraction with 1502.7µg/ml, as presented in Table 1. Hence, fraction F1 can be further utilized for investigation. Thus, fraction F1 was lyophilized and was kept for future use.

Estimation of carbohydrate and sulphate content

The lyophilized form of polysaccharide obtained after the purification process fraction (F1) was again subjected to estimation of carbohydrate and sulphate content to verify the efficacy of the extraction process after lyophilization. It was observed that the carbohydrate estimated from the purified fraction F1 was 1526.48 µg/ml and sulphate content gained was 70.4mg/ml. It can be further stated that purified polysaccharides showed promising results, and the presence of sulphate in the purified fraction confirmed the extraction process's efficacy. Therefore, it was confirmed that the lyophilized fraction obtained was sulphated polysaccharide as it reflected both carbohydrate and sulphate content and hence, can be used for further analysis. Subsequently, structural and chemical identification is an essential characteristic in the extraction process. Therefore, the purified sulphated polysaccharide was further subjected to structure prediction and investigation of the extracted polysaccharide's chemical profile to determine the chemical and functional groups present in it.

Structural Identification of Sulphated Polysaccharides

Prediction of the structure of the compound is a difficult job. Thus, to aid in this regard, Nuclear Magnetic Resonance spectroscopy is an efficient technique for

Table 1: Estimation of polysaccharide content in ion-exchange eluents

Sl. No.	Fractions obtained	Amount of Polysaccharide obtained (µg/ml)
1.	F1	1571.89
2.	F2	1286.48
3.	F3	1502.7
4.	F4	1167.56
5.	F5	1254.05

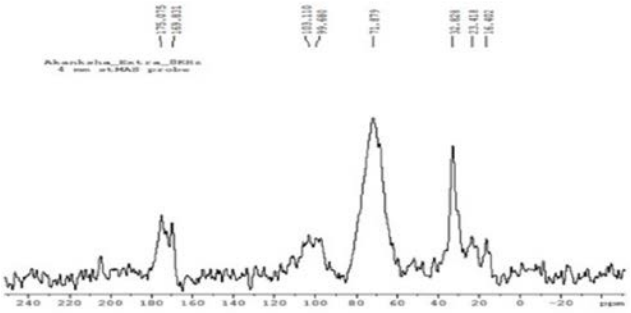

Figure 1: NMR spectrum of sulphated polysaccharide obtained from *D. bartayresiana*.

determining structures of all complex compounds, including sulphated polysaccharides.[19] The lyophilized sulphated polysaccharide was further subjected to solid-state Nuclear magnetic resonance spectroscopy analysis (NMR) with the aim that it can deliver essential information such as monosaccharide constituents, linkages, anomeric conformations, and loci of diverging or sulphated groups. It was found that these sulphated polysaccharides possess complex organizations, as displayed below in Figure 1. The results obtained from ^{13}C NMR revealed peaks at 175.075, 169.831, 103.110, 99.680, 71.879, 32.828, 23.418 and 16.402. The peaks achieved from the NMR investigation were similar to a probable polysaccharide fucoidan, the potential sulphated polysaccharide extracted from the particular algae. It was noted that the peaks obtained were highly correlated with fucoidan. The peak at 175.05 revealed the Amide / O–acetyl positions, peak at 169.8 revealed aromatic carbon ring, peak at 103.1 revealed unsaturated carbon or anomeric carbon, peaks at 71.8 revealed C≡C. In contrast, the peak at 32.8, 23.40 and 16.40 revealed sulphur group, saturated carbon, R_2-CH_2 or R-CH_3. The chemical groups obtained exposed all the probable components of fucoidan.

Identification of chemical profile of sulphated polysaccharide

After purification, the compounds obtained were lyophilized and subjected for the FTIR analysis that provided spectrum with elevated peaks at 3340.71, 2922.16, 1627.92, 1425.40, 1099.43 1032.13, 698.23, 619.5, 513.07 and 466.77^{cm-1}, respectively, as displayed in Figure 2. The peaks obtained demonstrated the presence of numerous organic and inorganic chemical compounds. Universally to every polysaccharide, the bands are observed in the stretch of 3,600–2,000 cm^{-1}. In contrast, the wideband at about 3,340 cm^{-1} indicated OH and H_2O distending vibrations, and quite a few minor bands at 2,922 cm^{-1} signified the CH stretching

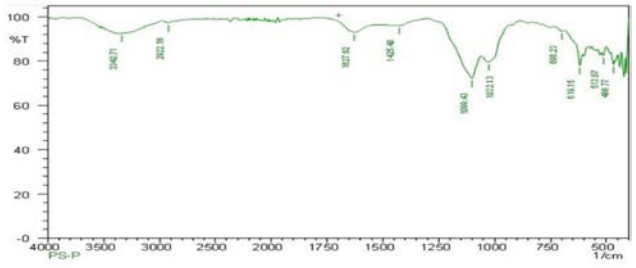

Figure 2: FTIR spectrum of sulphated polysaccharide.

Table 2: Estimation of Radical scavenging activity.

Sl. No.	Concentration of polysaccharide (µg/ml)	Inhibition Percentage (%)	
		DPPH assay	ABTS assay
1.	100µg/ml	1.1	24.6
2.	200µg/ml	2.9	24.8
3.	300µg/ml	4.1	27.6
4.	400µg/ml	5.2	29
5.	500µg/ml	5.8	29.8

in-ring and sulphur stretching. The peak at 1627 cm^{-1} reflected C=C stretch, which may be due to the fucose ring. Bands nearby 1,032 cm^{-1} also existed, indicating sulphoxide stretching. Penetrating bands at about 1,627 and 1,425cm^{-1} also existed and may be associated with uronic acid. The occurrence for the sulphated polysaccharide was validated in the fractions through peaks at 2922, 1627, 1032 and 1425cm^{-1}. The minor peaks at 698.23 cm^{-1} – 513 cm^{-1} may be consigned to alkyl halides stretching and vibrations. The location of the sulphate group is a significant asset of sulphated polysaccharides that regulate its structural and functional uniqueness. The results of FTIR were concordant with NMR results and indicated that the polysaccharide extracted was fucoidan.

Radical Scavenging Activity

The sulphated polysaccharide obtained was subjected to DPPH and ABTS assay to detect its radical scavenging activities. These assays revealed an increase in scavenging action at each concentration in both assays, and the results were displayed in Table 2. It was observed that the radical scavenging action was initiated at a minimal concentration of 100µg/ml. Further, a linear increase in radical scavenging activity was found from the minimal concentration of 100µg/ml concentration to the maximal concentration of 500µg/ml concentration. The percentage inhibition also displayed a remarkable increase ranging from 1.1% at 100µg/ml to 5.8% at 500µg/ml in the DPPH assay and from 24.6% to 29.85 in the case of ABTS assay in a dose-dependent manner. The purified polysaccharide, i.e. fucoidan extracted in our study, thus revealed virtuous radical scavenging actions. The IC_{50} value reported for the sulphated polysaccharide was 424.70µg in the case of DPPH assay and 186.43µg. It has been proved by our study that sulphated polysaccharide fucoidan possessed antioxidant properties.

Cytotoxic Effect of Fucoidan

The advancement of innovative drugs and remedial measures is essential to increase the chance of survival for patients detected with hepatocellular carcinoma. Natural compounds extracted from natural sources offer a variety of benefits due to their complex pharmaceutical applications. In our investigation, SK-Hep-1 cells, a human hepatic adenocarcinoma cell line, were treated with various concentrations of fucoidan ranging from 0.781µg/ml-250µg/ml, and cell viability was examined through MTT assay. Figure 3 displays the graph obtained for cell viability assay against Sk-Hep-1 cells treated with fucoidan. The SK-Hep-1 cells displayed the highest percentage of viable cells at a concentration of 0.781µg/ml with 51.53% cell viability, whereas only 7.85% cells were viable at 250µg/ml. The cell viability percentage decreased with the upsurge in the concentrations from 0.781µg/ml to 250µg/ml. These results, as mentioned earlier, indicate that the compound has cytotoxic effects against the liver cancer cell line Sk-Hep-1 cells. These cell lines revealed vast diversity in cellular uptake of drugs, owing to which they exhibited drug resistance. The inhibitory concentration (IC_{50}) value obtained was 30.6µg/ml. The treated hepatic adenocarcinoma cells look distorted with a shrunken appearance, whereas the control cells presented the distinctive feature of the corresponding cancer cell lines.

Detection of Apoptosis

To study the impact of fucoidan on apoptosis and evaluate cell apoptosis, Annexin V-FITC/propidium

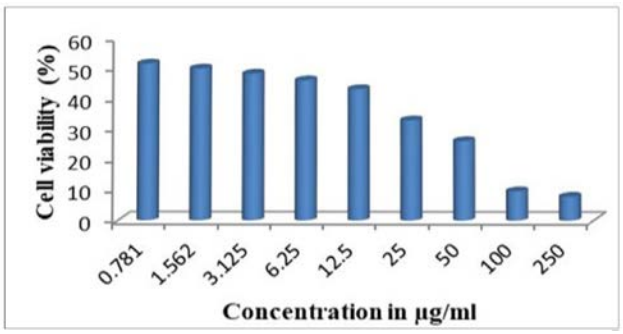

Figure 3: The graph obtained for cell viability assay against Sk-Hep-1 cells.

Table 3: Annexin fluorescent intensity at different concentrations

Sl. No.	Concentration of polysaccharide (µg/ml)	Fluorescent intensity
1.	Control	678.67
2.	12.5µg/ml	8898.33
3.	25µg/ml	9741.67
4.	50µg/ml	10889

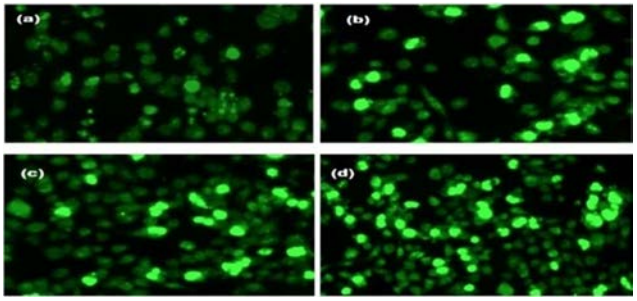

Figure 4: Fluorescent microscopic images of Annexin V-FITC/PI stained apoptotic cells (a) Control (b) cell lines treated with 12.5µg/ml fucoidan (c) cell lines treated with 25µg/ml fucoidan and (d) cell lines treated with 50µg/ml fucoidan.

iodide double-staining was performed and examined via fluorescence microscopy. The Sk-Hep-1 cells were nurtured with fucoidan particles at diverse concentrations (12.5µg/ml-50µg/ml) and were stained via Annexin V-FITC/propidium iodide double-stain. It was observed that to distinguish apoptosis, a hypersensitive probe checks the existence of phosphatidylserine on the membrane surface, as Annexin V fixes with phosphatidylserine owing to its high compatibility. Table 3 revealed the fluorescent intensities at different concentrations. The fluorescent intensity at control was 678.67. Similarly, 8898.33 at 12.5µg/ml, 9741.67 at 25µg/ml and 10889 at 50µg/ml, respectively, signifying apoptosis was tempted in the cells by fucoidan was dose-dependent. The results indicated that fucoidan initiated apoptosis in the cells. The images obtained from the assay are displayed in Figure 4, where (a) – (d) demonstrates the illustrative images of Annexin V/propidium iodide staining in Sk-Hep-1 cells at diverse concentrations of fucoidan. It was observed that no apoptotic cells were spotted in the control of SK-Hep-1 cells. In the images obtained from the apoptosis assay, it was found that the cells that bind to annexin V-FITC revealed radiance of green colour (FITC) on the cell surface (plasma membrane). The fluorescence in control cells was very weak, signifying negligible apoptosis. Fucoidan treated cell lines displayed a deep, bright apple green colour and increased dose-dependent at different concentrations ranging from 12.5µg/ml to 50µg/ml. Annexin-V-assay distinguished the early stages of apoptosis by tagging the peripheral cell membrane protein with phosphatidylserine (PS) in apoptotic cells and was discriminated from necrotic cells. Late apoptotic cells propidium iodide (PI) penetrated the cell membranes. At this stage, green fluorescent Annexin-V signals were observed because of the initial phase of apoptosis. Hence, it can be stated that the incidence of early apoptosis was indicative of the supplementary speedy and effectiveness of fucoidan treatment.

Detection of Autophagy

Autophagy initiation is demarcated by numerous ATG proteins, together with Atg5. The Atg5 protein is a part of the ATG autophagy controller family that regulates the extremely preserved cell's homeostatic reaction to an extensive assortment of self-and foreign-culminating cellular annoyances. Atg5 protein is far and wide articulated in numerous cells and localized using the cytoplasmic non-muscle actin protein beneath ordinary relaxing circumstances. Once the apoptosis initiates, Atg5 is then over-expressed, and Atg5 then forms a complex with other proteins of the Atg family and forms auto-phagosomes. The development of auto-phagosome occurs as a result of full-length Atg5. Therefore, western blotting was performed to detect autophagy and examine Atg5 protein levels in cells. From Figure 5, it was found that there was a rise in Atg5 levels in a dose-dependent manner. It was observed that after 24 hr of exposure of cells to fucoidan in different concentrations ranging from 12.5µg/ml to 50µg/mL, the level of Atg5 increased significantly in a dose-dependent manner and expression of β-actin remained constant at all concentrations. Both the β-actin and Atg5 were monoclonal with isotype IgG1 and IgG, respectively. The band size observed for

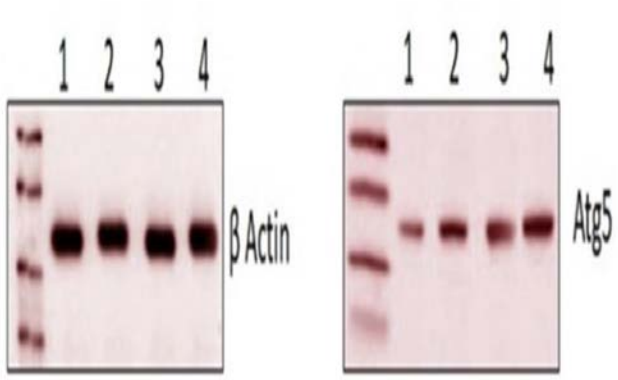

Figure 5: Western blot images revealing the bands of (a) β-actin and (b)-Atg5 at different concentrations grouped as 1-control, 2-12.5 µg/ml, 3-25 µg/ml, and 4-50 µg/ml.

β-actin was 42kDa, whereas, for Atg5, the band size was 55kDa, respectively. The results obtained displayed the Atg5 was present in Atg5-Atg12 conjugated form with a band size of 55kDa.

DISCUSSION

Since the sulphated polysaccharide have been known to possess several therapeutic advantages, numerous naturally occurring polysaccharides are acquired from natural sources like plants and algae, and increased research has been carried out in this area. Due to diversity in structure, these polysaccharides exhibit different biological properties among macromolecules. Previous studies demonstrated the anti-cancerous properties of fucoidan in diverse forms of cancers. Fucoidan regulates its action via several processes, including cell cycle arrest, immune system activation, and apoptosis. However, there was no clear evidence of activation of caspase 9 in the HAK-1B cell line, which is the human hepatic carcinoma cell line.[20] Therefore, in the existing study, we checked the occurrence of apoptosis in another human hepatic adenocarcinoma cell line, i.e. SK-Hep-1 cells. *Dictyota bartayresiana* is a brown alga commonly occurring in the tropical western Atlantic and central Pacific oceans. These algae possess numerous chemicals that are being investigated for potential use as antimicrobial. Fucoidan, the broad-spectrum word for sulphated polysaccharides, is known to be involved in countless biological events such as anti-tumour and anti-viral possessions, as stated above.[21] To our knowledge, the anti-cancerous effects of purified fucoidan extracted from *Dictyota bartayresiana* against hepatocellular carcinoma have never been tested before. Therefore, we attempted this study to evaluate the cytotoxicity, apoptosis and autophagy initiation by fucoidan in human hepatic adenocarcinoma cells. This study confirmed that fucoidan had a cytotoxic effect and encouraged apoptosis in adenocarcinoma cells. It was also confirmed that autophagy and apoptosis occurred in the fucoidan treated cells. Therefore, it can be stated that the fucoidan isolated from brown seaweed possessed the anti-cancerous properties against hepatic adenocarcinoma cells and the current study confirmed for the first time that fucoidan tempted apoptosis and autophagy in human hepatic adenocarcinoma cells SK-Hep-1 cells, and this study has paved the way for the development of drugs for curing cancers.

CONCLUSION

The objective of treating cancers is to eliminate tumour cells, preferably with marginal injury to healthy tissues. Since there are numerous side-effects of various current treatment methods, the usage of natural constituents of low toxicity is of concern. Since fucoidan also has immune-modulatory properties, it is hypothesized that it may have protecting properties against the growth of side effects when administered with different chemotherapeutic compounds and radiation. This report studied the fundamental cellular mechanism by which fucoidan tempts cell death within tumour cells. Hence, it can be concluded that the sulfated polysaccharide extracted from *Dictyota bartayresiana* was fucoidan. It exhibited efficient radical scavenging actions in both the assays. Fucoidan was found to suppress the cell propagation and cell viability of cells in a dose-dependent manner. Additionally, deep, bright apple green colour was also observed in fucoidan-treated SK-Hep-1 cells as an indication of Apoptosis. On the other hand, Western blot results for autophagy protein Atg5 was found up-regulated in fucoidan treated cell lines in a dose-dependent manner. In conclusion, the present study confirmed for the first time that fucoidan tempted Apoptosis and autophagy in human hepatic adenocarcinoma cell lines. Therefore, investigation of the anti-cancer mechanism of fucoidan will lead to a potent complementary drug for cancer treatment.

ACKNOWLEDGEMENT

The authors gratefully acknowledge VIT, Vellore, for the support through Seed Grant for Research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **BCL2:** B-cell lymphoma 2; **Da:** Dalton; **DAB:** 3,3'-Diaminobenzidine; **DEAE:** Sephadex-Diethylaminoethyl Sephadex; **DPPH:** 2,2-diphenyl-1-picryl-hydrazyl-hydrate; **F1-F5:** Fractions 1 to Fractions 5; **FITC:** Fluorescein isothiocyanate; **FTIR:** Fourier Transform Infrared Spectroscopy; **H_2SO_4:** Sulfuric acid; **IC_{50}:** half-maximal inhibitory concentration; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **NaCl:** Sodium Chloride; **NCCS:** National Centre for Cell Science; **NMR:** Nuclear Magnetic Resonance; **PBS:** Phosphate-buffered saline; **PI:** Propidium iodide; **PVDF:** polyvinylidene fluoride; **ROS:** Reactive Oxygen Species; **TNF:** tumour necrosis factor.

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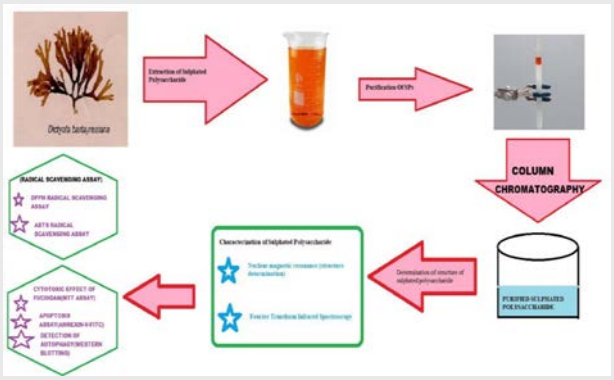
SUMMARY

The objective of the present study is to extract, purify, and characterize sulphated polysaccharides from *Dictyota bartayresiana* and evaluate its cytotoxic, apoptotic, and autophagy effects in human hepatic adenocarcinoma cell lines. The sulphated polysaccharide was extracted using a standard protocol and was purified via Column chromatography. Purification done by column chromatography yielded five significant fractions, which were further subjected to estimation of polysaccharide and sulphate content. In the existing study, it was found that the polysaccharide and sulphate content was found highest in fraction 1(F1) and was subjected to lyophilization. The lyophilized form of purified Sulphated polysaccharides obtained was subjected to NMR and FTIR for structural and chemical identification, confirming that the sulphated polysaccharide extracted was fucoidan. Assessment of the antioxidant and cytotoxic activity of fucoidan in human hepatic adenocarcinoma cell lines was performed. The results depicted that the antioxidant and cytotoxic effects increased in a dose-dependent manner. To study the impact of fucoidan on apoptosis and evaluate cell apoptosis, Annexin V-FITC/propidium iodide double-staining was performed and examined via fluorescence microscopy. The results obtained displayed an increase in fluorescent intensity at different concentrations signifying that apoptosis was tempted in cells by fucoidan in a dose-dependent manner. Additionally, deep bright apple green colour was also observed in fucoidan treated cells distinctive of cell apoptosis. Autophagy initiation is demarcated by ATG proteins, together with Atg5. Therefore, western blot was performed for Atg5 protein, and it was found that there was an increase in Atg5 levels in a dose-dependent manner. Thus, our study confirmed for the first time that fucoidan tempted apoptosis and autophagy in human hepatic adenocarcinoma cells.

PICTORIAL ABSTRACT



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Cite this article: Dubey A, Sivaraman J. Evaluation of the Cytotoxicity, Apoptosis and Autophagy Induction by Fucoidan in Human Hepatic Adenocarcinoma Cells. Indian J of Pharmaceutical Education and Research, 2022;56(3):756-64.