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Original Article

Chitin Derivative from Marine Source *Artemia franciscana* Cysts Inhibits Colorectal Adenocarcinoma Cells by Regulating Apoptotic Mediators

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ABSTRACT

Background: Chitin is widely preferred in biomedical application due to its biocompatible and biodegradable nature. Naturally occurring chitin is a potential antimicrobial agent and plays a major role in degrading outer shell wall of the lower crustaceans. Naturally occurring sources of chitin are more suitable for oncological research. **Objectives:** In the present study, the chitin derivative isolated from marine source (*Artemia franciscana* cysts) is evaluated for its potential to act as a therapeutic target for colorectal adenocarcinoma. **Materials and Methods:** The FTIR and X-ray diffraction analysis of the isolated chitin has confirmed the presence of characteristic functional groups such as amide groups, hydroxyl groups and glycosidic linkages along with their material's crystalline structure. **Results:** The anticancer activity of extracted chitin derivative showed significant cytotoxic effect in this study which could be attributed to their positively charged functional groups. Further mRNA gene expression analysis has revealed that the chitin derivative also exhibited apoptotic potential against HT29 cell lines by upregulating caspase-3 and caspase-9 gene and by down regulation of B cell lymphoma-2 (Bcl-2) gene expressions. **Conclusion:** In conclusion the present study showed that the *Artemia* sp. cyst-mediated chitin derivative could be a potential anticancer agent against colorectal adenocarcinoma through their anti-proliferative activity and by activation of apoptotic pathway in HT29 cell lines.

Keywords: Artemia, Cyst shell, Chitin, Anti-cancer, HT26.

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INTRODUCTION

Chitin is a linear polysaccharide complex chain formed by N-Acetyl D-glucosamine monomers and 1-4 glycosidic bonds.[1-2] Chitin is a most abundant natural polymer found in animals rather than in plant sources.[2] Typically chitin are found most abundant in the marine crustacean shells and studies have shown their primary function to be calcification process. They are also predominantly found in the cell wall of coralline algae, bacteria, brown and green algae. Chitin is generally preferred in biological applications because of their unique properties which include natural degradation, biocompatibility and nontoxic nature.[3] Chitin and chitosan are more preferred choices in pharmaceutical and oncological studies; more interestingly due to its low degradability in water, as efficient drug delivery systems and promote wound healing.[4-6] There is an ardent need for designing drugs which are of low toxicity while having only minimal side effect to normal cell line lineage.[7] Therefore, the present study is designed with an aim to provide an alternative yet efficient drug for cancer therapy utilizing the important potential of the chitin derivative.[8] Interestingly, chitin has active free amino acids chain

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with strong hydrogen bonded system through the crystalline structure, which makes them more biologically active and easily degradable.[9]

Most of the research has been carried out on chitin and its derivative's biological applications, particularly in its anti-angiogenic property, oncology, chemo-preventive effect. The ability of chitin and its derivatives to exhibit pro-inflammatory inhibition was reported in colon cancer by measuring different enzymatic activities like glutathione-S-transferase, quinine reductase, ornithine decarboxylase, cytokine-mediated nitric oxide, inhibition of heparanase and glutathione etc.[10-17] Similarly, anti-cancer property of chitin was investigated in human Hepatocellular carcinoma cell line (HepG2) and human ovarian cancer cell line (PA-1) and showed exciting findings.[16-17]The increased T-cell proliferation and suppressed cancer cell growth was reported in A549 (human lung), HepG2 (Human hepatoma) and PC3 (human prostate).[18] In the present study we aim to investigate the anticancer activity of purified chitin derivative on human colorectal adenocarcinoma cell line (HT-29).

MATERIALS AND METHODS

Sample collection

Artemia cyst shells were collected from Kelambakkam, Chennai, Tamilnadu, India. Samples were brought to the laboratory in brine solution (>200psu). Our earlier study has confirmed the presence of cysts from single species of brine shrimp *Artemia franciscana*.[19,20]

Extraction protocol for chitin

The cysts were segregated by bio-floating methods and healthy and unhealthy cysts were separated using sypinization. The unhealthy cysts were washed thrice using freshwater to remove debris found in the samples. The washed cysts were dried at 160°C in the hot air oven overnight. Finally, liquid nitrogen (N_2) was added to the dried samples to allow them to separate from hardest to easily breakable cyst shells. Finally, the dried cysts were ground to very fine powder using mortar and pestle.[21] The pulverized powder was demineralized by incubation with 1.5 M of dilute Hydrochloric Acid (HCl) for 30 min to remove the calcium carbonate and to stop hydrolysis of chitin present in the samples. For this process the samples were added in ratio of 1/30 (w/v) and stirred at room temperature at 150 rpm for 85 min.[21] The samples were then filtered through Whatmann filter paper No.1 and precipitate were collected. Then, the precipitate was washed through deionized water, again and dried at 80°C at overnight which helps to enhance the level of decalcification.

Finally, the demineralized powder was subjected to deproteination to remove the proteins. The powder was treated with 3M Sodium Hydroxide (NaOH) solution under continuous stirring for 85 min at 150 rpm. At the end of experiment sample was filtrated, washed and dried for generating possible chitin residues. This was followed by decolorization with acetone at a ratio of 1/10 w/v for 10 min at 150 rpm and filtered through Whatman filter paper. The precipitate was dried for 2 hr at room temperature. The obtained chitin residues were further treated with 0.315 M of sodium hypochlorite solution by 1/10 w/v for 10 min at 150 rpm. The finalized chitin product was filtered and dried in clean hot air oven. The purified chitin powder was kept in tightly vacuum-packed conditions.[20]

Analytical methods

The 1 mg pulverized final product of dried chitin powder and its derivatives was mixed with 100 mg of anhydrous KBr and subjected to IR spectra recording at laboratory conditions in the range of wavenumber from 400 to 4000 cm^{-n} (Perkin-Elmer Spectrum) with averaged resolution of 4 cm^{-1}. The crystallinity structure and atomic level of chitin was illustrated by X-ray Diffraction (XRD) analysis (Bruker AXS D8). Scintag powder diffracto meter was applied to elucidate between 2θ angles of 20° to 80° location. The structure of crystallinity of the obtained chitin polymer was evaluated by separating the area of appeared crystalline peaks and the total area covered under the curve range. 13C NMR was analyzed at 80°C with a Bruker Advance 300 spectrometer of 300 MHz equipped with 13C/1H dual probe. The 13C NMR spectrum was recorded with 3000 Hz and acquirement time of 1.36s; and a pulse width of 7s, with a relaxation time of 1s. The surface topography of isolated chitin was observed at maximized focus in Scanning Electron Microscopic (SEM) analysis with accelerated electron beam using JEOL, Model JFC-1600, Alagappa University, Tamil Nadu, India.[21-23]

Cytotoxic Activity of Chitin

Cytotoxicity assay of HT29

Initially, LC_{50} concentration was evaluated by using African green monkey kidney Vero cell line range from 25 µg/mL into 125 µg/mL. Based on MTT assay, the minimum and maximum concentration was fixed for cancer cell line of human colorectal adenocarcinoma (HT-29). Both cell lines were obtained as gift, from Department of Biochemistry, University of Madras, Tamil Nadu, India. These cells were maintained in DMEM supplemented with 10% of FBS (Fetal Bovine Serum), 2 mM of glutamine, 1.5 mg/mL of glucose and 100 UI cm^{-3} of penicillin and 100 g/mL streptomycin. For HT29 cell line the concentrations was fixed in the range of 10 µg/mL to 60 µg/mL. Then the cytotoxicity effect on HT29 was assessed by MTT assay. All the arithmetic average values were noted and documented.[24]

RESULTS AND DISCUSSION

Chitin is considered in biomedical application due to its biocompatibility and biodegradability nature.[25] Naturally chitin is a potential source for antimicrobial activity and resistance against natural elements which play major role in degrading outer shell wall of the lower crustaceans.[22] Without chitin many arthropods

might be eliminated from the ecosystem, therefore, naturally occurred valuable source of chitin is more suitable for oncological research.[21] There is need for optimization of concentration derived from chitin sources of different habitat.[26] We had extracted pure chitin from *Artemia* cysts by keeping its natural structure through standardized protocol of demineralization, deacetylation and deproteinization. As shown in Figure 1a the FTIR spectrum data for chitin, we observed the following trends, the wavenumbers ranges from 514.73 cm^{-1} to 3952.50 cm^{-1}. The transmittance values ranges from 75.94026549% to 119.0818584%. Some notable peaks and regions of interest in the spectrum include: A peak at around 1650 cm-1, which is typically associated with the carbonyl stretch in amides (C=O stretch). Peak at around 1550 cm^{-1}, corresponding to the amide II band (N-H bending and C-N stretching vibrations). A peak at around 1400 cm^{-1}, associated with the amide III band (C-N stretching and N-H bending vibrations). Peaks at the range of 1000-1300 cm^{-1}, representing the fingerprint region with characteristic chitin bands. A broad peak around 3300 cm^{-1}, indicating the presence of hydroxyl (O-H) stretching vibrations. As shown in Figure 1b, based on the provided FTIR spectrum data for chitin, here are some observations, the transmittance value ranges between 70 and 110, with some fluctuations throughout the spectrum. There are several distinct peaks and valleys in the spectrum that correspond to specific wavenumbers. The highest peak occurs at around 3750 cm^{-1}, indicating the presence of hydrogen bonding (O-H stretching vibration) in chitin. Other notable peaks include: A peak at around 1650 cm^{-1}, which corresponds to the amide I band and suggests the presence of C=O stretching vibrations of the amide group in chitin. Similar results strengthen the present observations by[27] the O-H group and N-H groups at the stretching of 3427 and 3257 cm^{-1} appearance with a peak value of 1377 cm^{-1} indicates the stretching of C-H bond. A peak at around 1550 cm^{-1}, which corresponds to the amide II band and indicates the presence of N-H bending vibrations and C-N stretching vibrations in chitin. Peaks in the region of 1050-1150 cm^{-1}, which correspond to the characteristic bands of C-O stretching vibrations in chitin.

The spectrum shows some variations in intensity and shape for different wavenumbers, indicating the presence of different functional groups and molecular vibrations in chitin. Overall, the FTIR spectrum confirms the presence of characteristic functional groups in chitin, such as amide groups, hydroxyl groups, and glycosidic linkages. It's important to note that a comprehensive interpretation of an FTIR spectrum requires additional analysis and comparison with reference spectra. The present study strongly agree with the earlier observations of Hisham *et al.*, 2021, who reported the peak values similar to present observations at 1652 and 1620 cm^{-1}, correspond amid groups at 1550 cm^{-1} indicated that alpha-chitin isomer. Based on the earlier observations of chitin and chitosan characterization through FTIR patterns with,[21,23] they reported excitation peaks of chitin at 1,650 and 1,620 cm^{-1} with the presences of amide group and similar findings were cited through 1,650 cm^{-1} peak range and they confirm the presence of

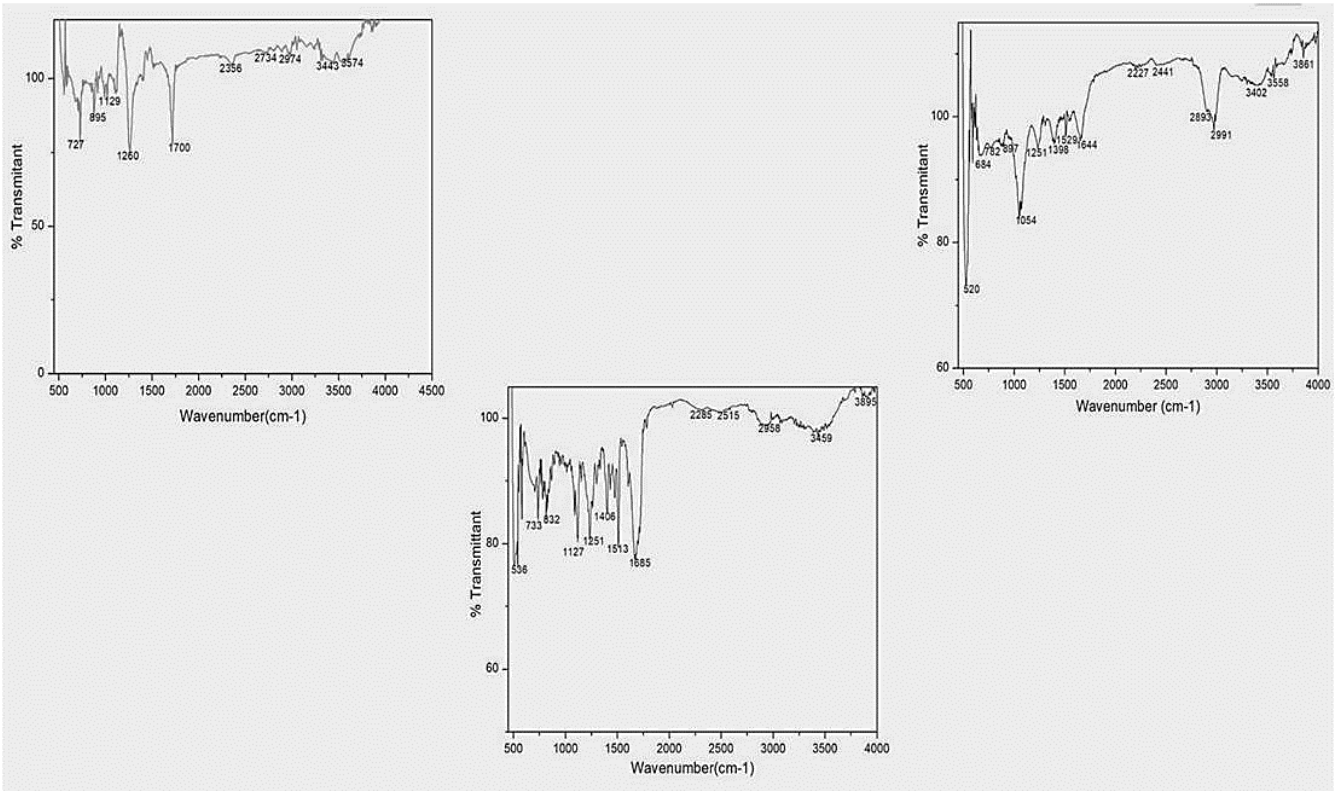

Figure 1: FT-IR spectrum of chitin and derivatives (a) Chitin, (b) and (c) low molecular weight chitin.

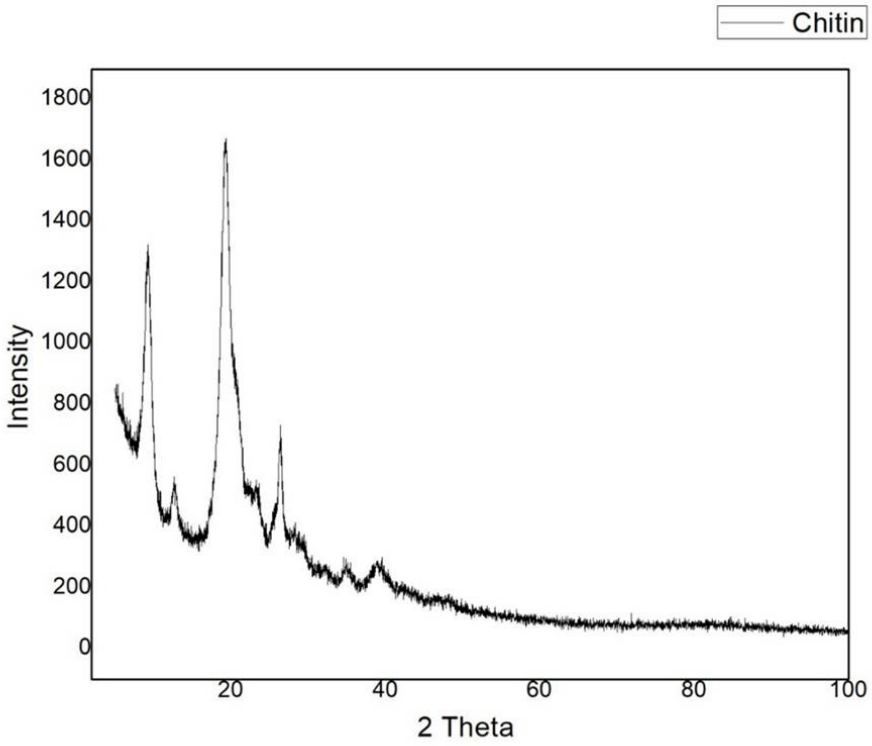

Figure 2: XRD analyses of chitin and their derivatives.

beta-chitin. Further the presence observations on hydrogen bond appearance in this study strongly correlated with the earlier study of,[34] The author also reported that the alpha crystal structure of chitin with similar hydrogen bonding in their extracted chitin derivatives. Further, the present observations strongly agreed with earlier chitin derivatives confirmation by FTIR patterns of crustacean shells of different organisms.[28-31]

The above observations provide a general overview based on the given data, but a more detailed analysis may be necessary for a complete characterization of the chitin sample. As shown in Figure 1c, the spectrum of low molecular weight chitin might exhibit peaks at similar wavenumbers as regular chitin, the intensities and shapes of these peaks could be different due to the altered molecular structure. The peak intensities might vary compared to regular chitin due to the altered composition and arrangement of functional groups. For instance, the intensity of the carbonyl (C=O) stretching peak might differ, reflecting changes in the amide content. Low molecular weight chitin might exhibit shifts in peak positions or broader peaks, indicating changes in hydrogen bonding patterns or altered crystallinity. The fingerprint region (900-1200 cm^{-1}) might show changes in peak patterns due to variations in glycosidic linkages and ring vibrations in low molecular weight chitin.

To justify that the observed FTIR spectrum corresponds to low molecular weight chitin, it was compared with the observed peaks and patterns with published FTIR spectra of low molecular weight chitin in the literature.[28-31] The FTIR spectrum of chitin exhibits characteristic peaks in the fingerprint region (900-1200 cm^{-1}) associated with glycosidic linkages and ring vibrations. Strong peaks around 1650 cm^{-1} indicate amide I vibrations, while peaks around 1550 cm^{-1} correspond to amide II vibrations. The spectrum suggests the presence of chitin's typical molecular structure. The FTIR spectrum of low molecular weight chitin type 1 shows shifts in peak positions and alterations in peak intensities compared to regular chitin. These changes could be attributed to the modifications in the molecular structure or arrangement of functional groups due to the low molecular weight nature.[32] The overall pattern of peaks is still indicative of chitin. The FTIR spectrum of low molecular weight chitin type 2 also displays shifts and variations in peak intensities, similar to type 1. These changes may reflect the altered structure and composition associated with low molecular weight chitin. Despite these variations, the general spectral features remain consistent with chitin.[32]

In this study, FTIR spectra were collected for three different samples: chitin, low molecular weight chitin type 1, and low molecular weight chitin type 2. The FTIR spectrum of chitin exhibited characteristic peaks corresponding to glycosidic linkages, amide I, and amide II vibrations, confirming the presence of chitin's molecular structure.[32] The spectra of low molecular weight chitin types 1 and 2 displayed shifts and alterations in peak intensities compared to regular chitin, likely due to changes in molecular arrangement caused by their lower molecular weight.[32,33] Despite these variations, the spectra of all three samples showed distinct patterns consistent with chitin,

indicating the presence of chitin-like structures. These results suggest that even in the context of low molecular weight chitin, the fundamental molecular features characteristic of chitin are preserved, albeit with some modifications.

In this study, the X-ray Diffraction (XRD) results provide valuable insights into the material's crystalline structure, particularly in relation to chitin. Chitin, a prominent biopolymer characterized by a distinct crystalline lattice, exhibits characteristic diffraction peaks in its XRD pattern shown in Figure 2. Our observed data reveals peaks at approximately 2θ values of 9°, 20°, and 26°, aligning closely with the anticipated positions for chitin. These peaks are indicative of a crystalline arrangement resembling chitin's repeating units of N-acetylglucosamine.[34,35] The intensity of observed peaks is consistent with chitin's known intensity distribution. Additionally, the presence of minor peaks at different angles further supports the correlation to chitin. While the level of crystallinity can vary, our relatively sharp and well-defined peaks suggest a notable degree of order within the material.[36] These findings are substantiated by the sample's origin, sourced from chitin-rich biological materials. The presence of sharp and well-defined peaks within the 2θ range of 20° to 80° indicated a high degree of crystallinity, suggesting that the chitin maintained

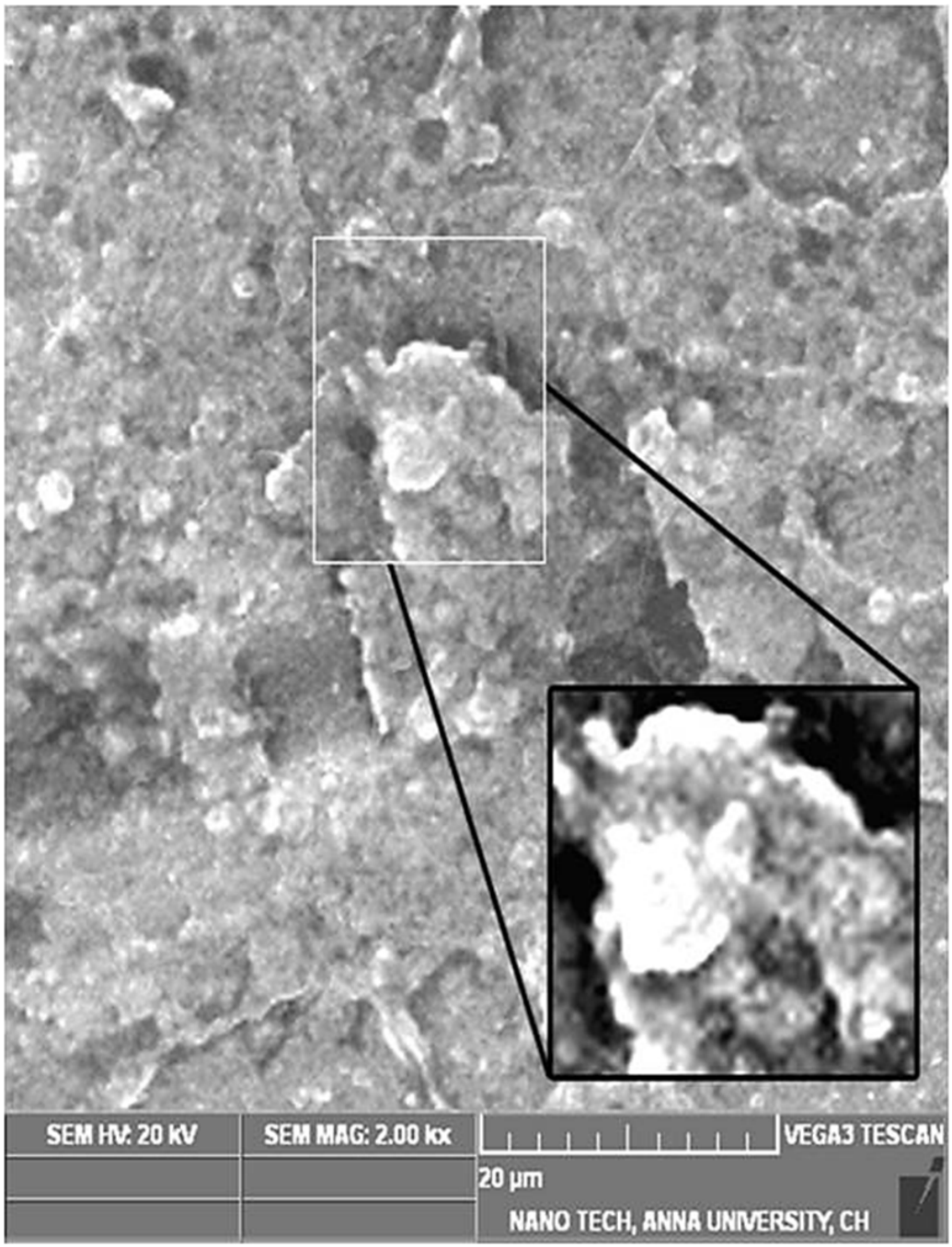

Figure 3: Surface topography and nature of low molecular weight chitin derivatives.

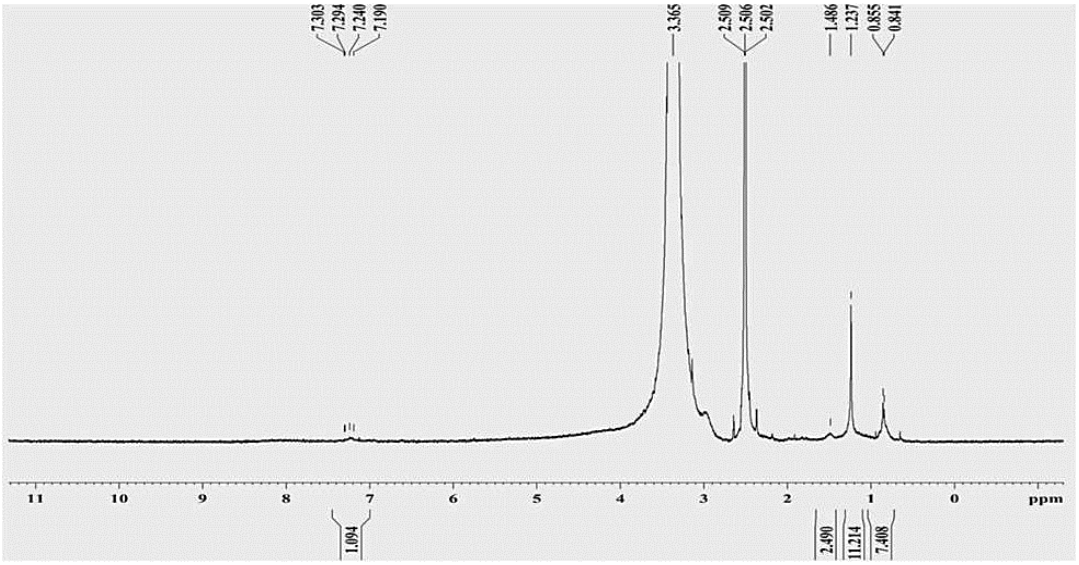

Figure 4: H1 NMR spectrum of low molecular weight chitin.

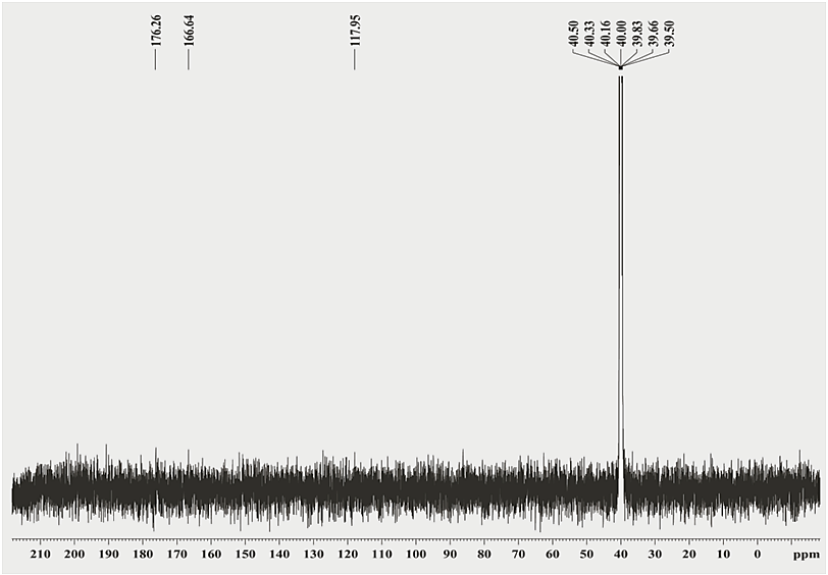

Figure 5: 13C NMR spectra of low molecular weight chitin derivatives.

its native polymeric structure. A high CrI value is indicative of effective deproteinization and demineralization, with minimal amorphous content remaining. This degree of crystallinity reflects the structural integrity and purity of the chitin, making it suitable for applications in biomedical and pharmaceutical fields. To augment the identification, future investigations could encompass complementary analyses, such as Fourier-transform infrared spectroscopy and microscopy, to corroborate the presence of chitin. The robust alignment of our XRD data with published references of chitin patterns underscores the likelihood of chitin's presence within the analyzed material.[23,34,35,37]

The surface topography of chitin was shown in Figure 3, revealed that different very dense with tightly packed. Earlier, we were observed the similar structure with minimized magnification shown in[34] and that was shown in very hard nature with pellet-like structure. Based on the observations process of extraction of chitin makes differences in the solid structure formation. This could help to understand the mechanisms in which involved biological activity in the partially diluted 10% ortho phosphoric acid chitin.[38] This structure similar with study who extracted chitin from Millipede (Spirobolida) and characterized through FTIR and SEM analyses, this study obtained around 35.7% of yield by conventional sources.

Further, the purity of chitin isolated from *Artemia* cysts waste was evaluated through 13C H_1 Nuclear Magnetic Resonance (NMR) spectroscopy as shown in Figures 4 and 5. The identical spectrum was noted at each spectrum indicates glucosamine ring at 50 and 110 ppm, indicated that high homology found within the structure. The 173 ppm indicating that, corresponding signal found to C=O groups with conformational state in the isolated chitin and 23 ppm indicating methyl groups. The characteristic situation of chitin was noted through the signal found in the C3

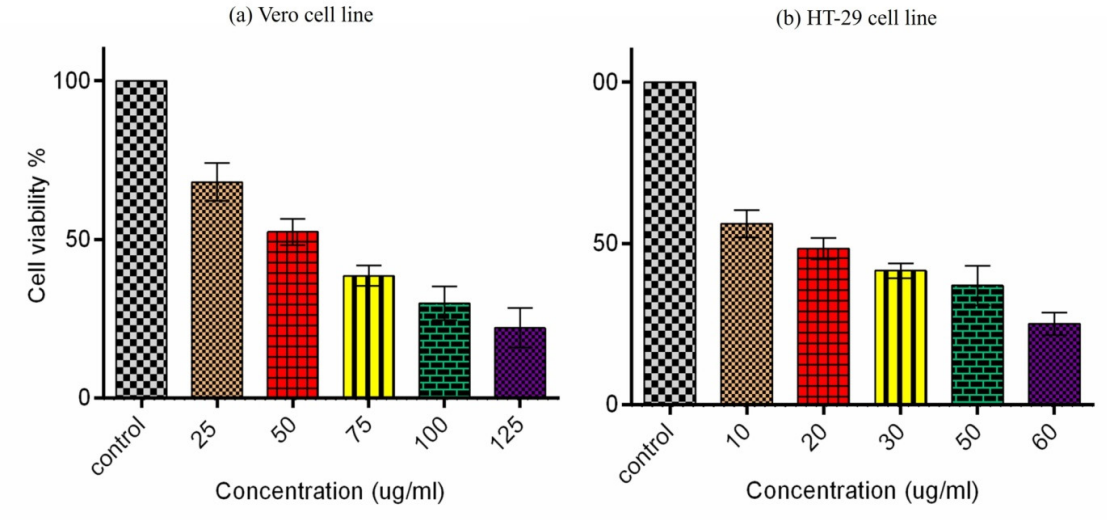

Figure 6: Cytotoxic activity of chitin with various concentrations against HT-29 cell line compared with Vero cell line. (a) Vero cells (b) HT-29 cells.

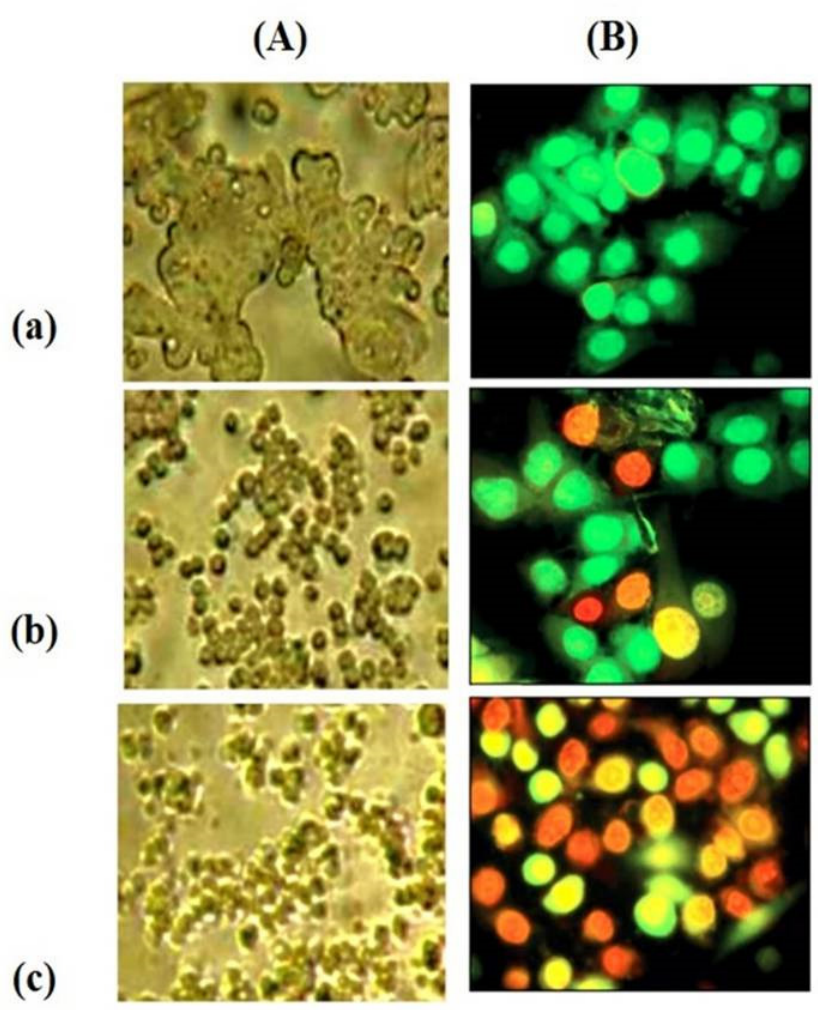

Figure 7: Fluorescent micrographs of Acridine Orange/Propidium Iodide Double-Stained HT-29 Cells demonstrating chitin's cytotoxic activity at varied concentrations. (a) Control cells untreated (b) Cells treated with chitin 25 µg/mL (c) Cells treated with chitin 75 µg/mL.

at 76 ppm and C5 at 74 ppm. The deacetylation, deproteinization and demineralization indicating that prepared chitin might be isomorphic with solid state with confirmation of SEM.

With reference to anticancer activity of extracted chitin showing significant cytotoxic and apoptotic effect against to HT29 cell lines. The results elucidated that the chitin at the concentrations of 50 µg/mL with maximized cell death of 63.27% (Figure 6). The concentration of 50 µg/mL indicates potential level drug treatment to the HT29 cell lines without any impact to the normal cell lines lineages. The isolated chitin showed a potential anti-proliferative effect with maximized cytotoxicity in the HT29 cell lines with increased concentrations. The present observation strongly correlate with earlier observation of who reported 400 µg/mL concentration had a maximized cytotoxicity in the Hep2 human cancer cell lines. The cytotoxic effect strongly contributed through chitin and cancer cells by accelerating positively charged functional groups of chitin with negatively charged cellular components in the tumor cells. Further, structure related biding sites on the cancer cell lines may suggest the hidden molecular mechanisms of cellular interaction between the chitin and tumor cell lines.[39] Interestingly, study of Baouche *et al.*, 2014 revealed, chitin act as bio adhesive nature with strongly interacting with surface glycoproteins of tumor cell lines. This specific characteristic feature of chitin helps to disintegration lipid bilayers of targeted cell lines.[40]

The cytotoxic activity of chitin with different concentration against HT-29 and vero cell lines was evaluated. From the data, we can observe that as the concentration of chitin increases, the cell viability of Vero cells decreases. This indicates a cytotoxic effect of chitin on Vero cells. The lower the cell viability percentage, the higher the cytotoxicity. Similarly for the cytotoxic activity of chitin with HT-29 cell line: we observe a decrease in cell viability of HT-29 cells as the concentration of chitin increases. This suggests that chitin exhibits cytotoxicity against HT-29 cells as well (Figure 7).

The metastasis inhibition of chitin in the human lung and liver cancer cell lines was reported *in vivo* model of mice,[41] these findings are further supported by the study of Luan *et al.*, 2022 who suggested that the sulfated chitin enhance the anti-inflammatory effect on tumor cell lines through its phosphorylated glucosamine functional groups.[42] To compare the cytotoxic activity of chitin between Vero and HT-29 cell lines, the cell viability percentages for different chitin concentrations were plotted on a graph. The cell viability of Vero cells decreases as the concentration of chitin increases.[41] At a concentration of 25 µg/mL, the cell viability is around 68%, and it decreases to approximately 22.5% at a concentration of 125 µg/mL. The cell viability of HT-29 cells also decreases with increasing chitin concentration. At a concentration of 10 µg/mL, the cell viability is approximately 55.9%, and it decreases to about 24.9% at a concentration of 60 µg/mL. comparing the two cell lines, we can see that both Vero and HT-29 cells exhibit reduced cell viability in the presence of chitin. However, it appears that HT-29 cells are more sensitive to the cytotoxic effects of chitin compared to Vero cells.[43] At lower concentrations, HT-29 cells already show a lower cell viability compared to Vero cells. Based on these findings, we can conclude that chitin demonstrates cytotoxic activity against both Vero and HT-29 cell lines. However, HT-29 cells exhibit higher susceptibility to the cytotoxic effects of chitin compared to Vero cells. It is important to note that this conclusion is based on the provided data and further studies may be needed to evaluate the mechanism of chitin's cytotoxicity and its potential applications in cancer research or therapeutic interventions.[43]

After the experiment with 48 hr of incubation the HT-29 cells were subjected to the expression the mentioned gene in the treated cell line compared with housekeeping gene expression of beta-actin. mRNA expression analysis showed upregulation of Caspase-3 and Caspase-9 while anti-apoptotic gene Bcl-2 was downregulated. The observed initiation of apoptosis in HT29 cancer cells by the chitin derivative suggests the involvement of the intrinsic (mitochondria-mediated) apoptotic pathway. This pathway is primarily regulated by the balance between pro-apoptotic and anti-apoptotic proteins of the Bcl-2 family. In our study, the significant upregulation of caspase-9 and caspase-3 gene expression, and with downregulation of Bcl-2, indicates a disruption in mitochondrial membrane integrity, that leading to

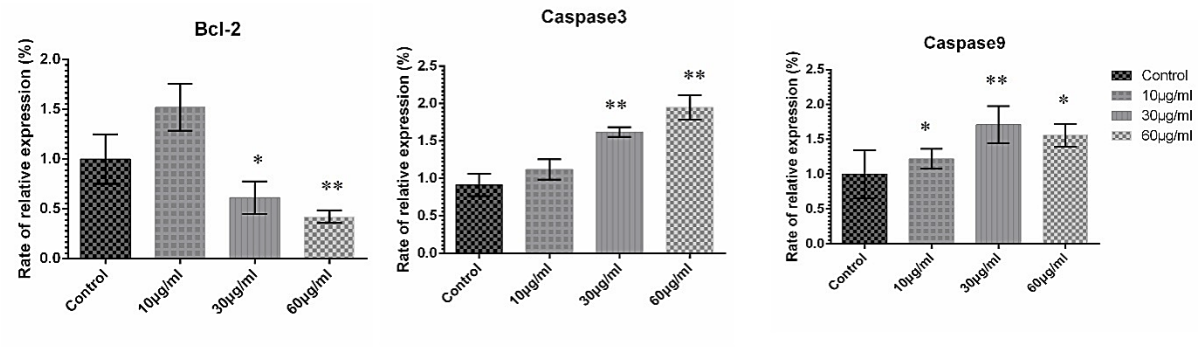

Figure 8: Real Time RT-PCR data show level of Bcl-2, Caspase-3 and Caspase-9 gene expression in response to treatment after 48 hr of incubations with various concentrations of chitin.

the release of cytochrome c into the cytoplasm. Therefore this release forms an apoptosome complex that activates caspase-9, which in turn activates caspase-3, culminating in programmed cell death. The suppression of Bcl-2 further enhances mitochondrial permeability, facilitating this cascade. These molecular events confirm that the chitin derivative does not exert cytotoxic effects but initiates a regulated apoptotic mechanism as observed in Figure 8, therefore, the minimum inhibitory (10 µg/mL) and maximum inhibitory (60 µg/mL) concentrations of the chitin had a potential source of anti-proliferative and anti-inhibitory effect against HT-29 cell lines. The effect of chitin and their derivatives on HT-29 cell line was strongly authenticated by Li *et al.*, 2016 and Rhoades *et al.,* 2006, they reported that the chitin and their derivatives are being used in biomedical application due to their biodegradable and biocompatible property. The present observations strongly suggest that *Artemia* sp. cyst-mediated chitin has potential as an anticancer agent for the HT-29 cell line to inhibit their proliferation and enhance the apoptotic-related gene.

CONCLUSION

Around the world many of uncared salterns are rapidly wasted which are a promising source of *Artemia* cysts from which a huge amount of chitin could be isolated. All the healthy cysts are damaged by many ecological enemies like ants, beetles and weathering soils. Those abandoned unhealthy cysts from wasted shrimp shells are ethically valuable sources for chitin extraction. Each year, several aquaculture industries discard plenty of unhealthy cysts. Hence the present study is mainly focused on usage of locally sourced unhealthy *Artemia* cysts for biomedical application without affecting the environment. In the present study we have reported the use of isolated chitins derived from unhealthy cysts of wasted shrimp shells which are highly pure and characterized by FTIR, XRD and NMR spectroscopy analyses. Further, the surface topography and nature chitin were observed by SEM electrophotography. With reference to biomedical application, we have examined the potential anti-cancer activity of isolated chitin to target tumor cell line HT29 with minimized level of cytotoxicity effect. Further, we have elucidated apoptotic activity of chitin through different EtBr/AO staining procedure. The maximized level of apoptosis activity was at 50ug/mL concentration. Therefore, *Artemia*cyst shell derived chitin could be a promising source of anti-proliferative and anti-cancer property.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectroscopy; **Bcl-2:** B cell lymphoma-2; **HepG2:** Human hepatocellular carcinoma cell line; **PA-1:** Human ovarian cancer cell line**; HCl:** Hydrochloric acid; **NaOH:** Sodium hydroxide; **XRD:** X-ray diffraction; **SEM:** Scanning electron microscopy.

AUTHOR CONTRIBUTION

PR and TS contributed equally throughout this study. SJS, JA, LK, CJMD, RN, VPV, HJS, KMS and RRR were involved in designing the objective, work plan, experimental analysis, data interpretation, drafting the article and final approval of the manuscript.

SUMMARY

The study investigates the potential anticancer properties of chitin derived from *Artemia franciscana* cysts against colorectal adenocarcinoma. The extracted chitin was characterized using FTIR, XRD, and NMR spectroscopy, confirming the presence of key functional groups and crystalline structures. Cytotoxicity assays demonstrated significant anti-proliferative effects on HT-29 cancer cells, with upregulation of apoptotic markers Caspase-3 and Caspase-9 and downregulation of the anti-apoptotic gene Bcl-2. The findings suggest that Artemia cyst-derived chitin could serve as a promising natural anticancer agent.

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