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Bacillus sonorinses derived exopolysaccharide enhances cell cycle arrest, apoptosis, necrosis, autophagy and COX-2 down regulation in liver cancer cells

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is one of the most serious types of cancer that accounts for numerous cancer deaths worldwide. HCC is poorly prognosed and is a highly chemotherapy-resistant tumor. Therefore, new treatments are urgently needed. Exopolysaccharides (EPS-1) produced from the novel *Bacillus sonorensis* strain was found to exhibit chemopreventive effects against cancer.
Objective: Evaluating the anti-cancer cytotoxic effect of exopolysaccharides (EPS-1) produced by the newly studied *Bacillus sonorensis* strain SAmt2.
Methods: The cytotoxic activity was investigated through cell cycle, apoptosis, and autophagy analyses using flow cytometry technique. Also, the effect of EPS-1 on Huh7 release of COX-2 was examined using ELISA.
Results: Our results revealed that EPS-1exhibit an anti-proliferative effect on Huh7 cells through decreasing the percentage of cells at the S-phase and G2 phase, while increasing the cell population at the sub-G1 and G1 phases. Apoptosis analysis showed that EPS-1 increased necrotic and apoptotic cell fractions in EPS-1 treated Huh7. In addition, it induced significant autophagic cell death in the Huh7.Finally, antiproliferative and apoptosis induction results were supportedby ELISA assay results where the protein level of COX-2 was declined.
Conclusion: : In conclusion, EPS-1 derived from *B. sonorensis* SAmt2, is a promising proliferation inhibitor of Huh7 cells with potential anticancer effects.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer; one of the most serious health problems and a leading cause of mortality and accounts for approximately 90 % of liver cancer cases [1]. HCC is a malignancy that arises from the uncontrolled proliferation of hepatocytes [1,2]. Relative to other malignancies, liver cancer is poorly prognosed in early stages, liver transplantation is the recommended treatment, while surgical resection, radiofrequency ablation and chemoembolization are the approved treatments for advanced HCC [2]. Standard chemotherapy has short response duration, an elevated recurrence rate, and a declined patient survival rate [3]. In addition to being a highly chemotherapy-resistant tumor, most HCC chemotherapy regimens are greatly limited due to the underlying liver disease [4]. Moreover, existing chemotherapeutic agents have a number of drawbacks, including, toxic side effects, therapy resistance and insufficient efficacy in advanced cancers [2]. For example, Doxorubicin increases NF-κB activation through the formation of free radicals or reactive oxygen species (ROS) that can damage cells non-selectively causing nephrotoxicity and cardiotoxicity [5].

Liver chronic inflammation is a recognized risk factor for HCC as a later manifestation of a prolonged inflammatory process may lead to genetic changes that could represent an early step in the development of malignancy [6]. Cyclooxygenase-2 (COX-2), one of the mediators of the inflammatory pathways, may contribute to tumor formation. COX-2 expression is undetectable in normal tissues, and is highly induced by

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pro-inflammatory mitogens, cytokines, growth factors and tumor promoters. It is now well-established that COX-2 is chronically over-expressed in many premalignant, malignant, and metastastic cancers, including hepatocellular carcinoma [7]. In tumors, over-expression of COX-2 leads to an increase in prostaglandin (PG) levels, which affect many mechanisms, involved in carcinogenesis, such as inhibition of apoptosis, stimulation of cell growth besides the metastatic potential and invasiveness of tumor cells. Multiple evidence indicated that COX-2 is an important molecular target for anticancer therapies, and COX-2 inhibitors appear to have anticancer effects in different types of malignancies including liver cancer [7]. It is important to find out a co-treatment against these toxic effects, along with targeting cancer cells proliferation. Figuring out the best regimen and use of agents to suppress or even alleviate the side effects of chemotherapy is an interesting challenge [8]. Moreover, targeting cell cycle inhibition as well as promoting apoptosis is one of the essential ways to disturb cancer development [7,8].

Exopolysaccharides (EPS) are metabolic by-products of microorganisms. They are extracellular macromolecules excreted as tightly bound capsule or loosely attached slime layer in microorganisms such as bacteria. They represent safe value added substances that exhibit different biological activities in vitro and in vivo [9]. EPS produced from *Bacillus* strains have been reported to have multiple beneficial biological functions for human health.They have antioxidant and free-radical scavenging activity [9,10], hydroxyl radical scavenging activity [11], anti-UV protective activity [12], blood glucose regulating activity [13], immunological activity [14], antiviral and immuno-regulatory activities [15], and importantly, anti-cancer activity [16–18]. It was demonstrated that bacterial secondary products can greatly attenuate cancer development [19].The novel *Bacillus sonorensis* strain has an enhanced ability to produce large quantity of extracellular polysaccharides [20]. The EPS produced from the novel *B. sonorensis* strain exhibit various anti-cancer and chemopreventive effects [18,21].

In the current study, we aimed to extend our evaluation of the anticancer effects of EPS-1 isolated from *B. sonorensis* strain SAmt2, which was previously isolated from the Egyptian Mediterranean Marsa matrouh sea sediment (its 16S rRNA partial gene sequence has been recorded in the NCBI Gene Bank under accession number KP733899). Our previous study revealed an inhibitory influence for *B. sonorensis* strain SAmt2 EPS-1 on Huh7 and HepG2 cell growth as well as an up-regulation p53 gene [18]. Herein, we studied cytotoxic activity of *B. sonorensis* EPS-1 on Huh7 cells through cell cycle arrest autophagy and apoptosis analyses using flow cytometry technique, in addition to examining the protein secretion of COX-2.

2. Material and methods

2.1. Production, isolation, and purification of EPS-1

The bacterial isolates were stimulated to produce EPS-1, then the EPS-1 were isolated and purified according to our previously published method [18]. Briefly, EPS-1 was purified through centrifugation followed by treatment with tricloro acetic acid and finally it undergone dialysis. Chemical analysis of the purified EPS was performed by the phenol-sulfuric acid method as described before [18,22].

2.2. Cell culture and expansion

Huh7 cell line used in this study was purchased from ATCC (American Type Culture Collection) and maintained in the proper conditions. Cells were cultured in the presence of a complete culture media consisting of Dulbecco's modified Eagle's Medium (DMEM) (Lonza, Beligium) supplemented with 10 % fetal bovine serum (FBS), 4 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere with 5 % CO2 concentration. Media were exchanged twice a week. Upon reaching 80 % confluence, cells were harvested by trypsinization (0.025 % trypsin and 0.02 % EDTA) and propagated to the next passage.

2.3. Cell cycle analysis

Huh7 cell cycle analysis was performed using flow cytometry as described by Fekry et al. [23]. Huh7 cells were treated with *B. sonorensis* SAmt2 EPS-1 for 24, 48, and 72 h. Huh7 cells cultured without the addition of any treatments were used as the negative control. Cells (10^5 cells) were harvested by trypsinization and washed twice with ice-cold PBS (pH 7.4). For fixation, cells were re-suspended in 2 ml of 60 % ice-cold ethanol and incubated for 1 h at 4 °C. Fixed cells were washed twice again with PBS (pH 7.4) and resuspended in 1 mL of PBS containing 50 μg/mL RNAase A (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and 10 μg/mL propidium iodide (PI) (BD Biosciences). Cells were incubated in dark for 20 min, then after incubation DNA contents of cells were analyzedby flow cytometry using FL2 (λex/em 535/617 nm) signal detector (ACEA Novocyte™ flowcytometer, ACEA Biosciences Inc., San Diego, CA, USA). For each sample, 12,000 events were acquired. Cell cycle distribution was calculated using ACEA NovoExpress™ software (ACEA Biosciences Inc., San Diego, CA, USA).

2.4. Cell apoptosis analysis by flow cytometry

The apoptotic and necrotic cell populations were determined using flow cytometry as previously described by Fekry et al. [23]. Annexin V-FITC apoptosis detection kit (Abcam Inc., Cambridge Science Park, Cambridge, UK) was used coupled with 2 fluorescent channels flowcytometry. Huh7 cells were treated with *B. sonorensis* SAmt2 EPS-1for 24, 48, and 72 h. Huh7cells cultured without the addition of any treatments were used as the negative control. Cells (10^5 cells) wereharvested by trypsinization, washed twice with ice-cold PBS (pH 7.4), then incubated with 0.5 ml Annexin V-FITC/PI solution for 30 min in dark at room temperature. After being stained, Huh7 cellswere injected into ACEA Novocyte™ flowcytometer (ACEA Biosciences Inc., San Diego, CA, USA) and analyzed for FITC and PI fluorescent signals using FL1 and FL2 signal detector, respectively (λex/em 488/530 nm for FITC and λex/em 535/617 nm for PI). For each sample, 12,000 events were acquired. Positive FITC and/or PI cells were quantified by quadrant analysis and data were analyzed by ACEA NovoExpress™ software (ACEA Biosciences Inc., San Diego, CA, USA).

2.5. Autophagy analysis

To furtherly determine the method of cell death by of liver cancer cells in response to EPS-1 treatment, autophagic cell death was quantitatively measured via flowcytometry autophagic assay as described by Bashmail et al. [24].Using acridine orange lysosomal stain. Huh7 cells were treated with *B. sonorensis* SAmt2 EPS-1 for 24, 48, and 72 h. Huh7 cells cultured without the addition of any treatments was used as the negative control. Cells (10^5 cells) were harvested by trypsinization, washed twice with ice-cold PBS (pH 7.4), then incubated in dark for 30 min with acridine orange (10 μM). After being stained, cells were injected into ACEA Novocyte™ flowcytometer (ACEA Biosciences Inc., San Diego, CA, USA) and acridine orange fluorescent signals were analyzed using FL1 signal detector (λex/em 488/530 nm). For each sample, 12,000 events were acquired and positive FITC and/or PI cells were quantified by quadrant analysis and calculated using ACEA NovoExpress™ software (ACEA Biosciences Inc., San Diego, CA, USA).

2.6. Enzyme-linked immunosorbent assay (ELISA)

Quantification of secreted COX-2 protein in cell supernatants was performed using ELISA analysis according to Abu-Shahba et al. [25]. Conditioned media (CM) of cultured EPS-1 treated Huh7 cells and untreated Huh7 cells were collected, after incubation for 48 h, and stored

at -80 °C for later analysis. The concentration of secreted COX-2 was detected using a quantitative human ELISA Kit (Biovision, ABCAM, US) according to the manufacturer's instructions.

2.7. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using the IBM Statistical Package for the Social Sciences software (SPSS) version 25 (IBM SPSS Statistics 25, IBM Corp. NY, US). Normally distributed data were analyzed usingun-paired Student's t-test while data with non-Gaussian distribution were analyzed using non-parametric Mann-Whitney test U nonparametric test. $p < 0.05$ was considered as a cut-off value for significance.

3. Results

3.1. Morphology of EPS-1 treated Huh7 cells

EPS-1 treated Huh7 cells were examined under the microscope to follow up any changes after 24, 48, and 72 h. Microscopical examination showed no apparent changes in morphology or cell size, however, EPS-1 treated Huh7 exhibited lower cell confluency after 48 and 72 h as indicated by the empty spaces relative to untreated cells which exhibited more confluency (Fig. 1). The lower confluency denotes the decline in the proliferative ability of treated Huh-7 cells in comparison to their untreated counterparts.

3.2. Effect of B. sonorensis SAmt2 EPS-1 on the cell cycle phases of Huh7liver cancer cells

We examined the impact of *B. sonorensis* SAmt2 EPS-1 on the cell cycle of Huh7 cells using fluorescence-activated cell sorting technique, after exposure of cells to EPS-1 for 24, 48, and 72hours (Fig. 2).Cell cycle analysis revealed that SAmt2 EPS-1 induced a significant increase in cell population at the G1 phase and Sub-G1 (G0/G1) phase denoting non-proliferating cells after 48 h (p-value < 0.01 and <0.05 respectively) and 72 h (p-value < 0.01). Meanwhile, EPS-1 induced a significant decrease in the proportion of Huh7 cells at the S phase after 48 and 72 h of exposure (p-value < 0.01 and <0.001 respectively) indicating a depletion in the number of dividing cells. Also, a decline in the number of cells at the G2 phase was observed after 24, 48, and 72 h post treatment (p-value < 0.01). These results reflect the inhibitory effect of *B. sonorensis* SAmt2 EPS-1 on the proliferation ability of liver cancer cells.

3.3. Effect of B. sonorensis SAmt2 EPS-1 on Huh7 liver cancer cell apoptosis

The degree of cell apoptosis in response to treatment with *B. sonorensis* SAmt2 EPS-1 was analyzed using Annexin V-FITC/PI double staining. *B. sonorensis* SAmt2 EPS-1induced an increased percentage of early-stage apoptotic cells after exposure for 48 (p-value $= 0.05$) and 72 hourscompared with the untreated Huh7 cells with no significant p-value. While the percentage of necrotic cells was significantly increased after the three time points (p-value < 0.01, <0.05, <0.01 respectively) showing a necrosis enhancing impact for the *B. sonorensis* SAmt2 EPS-1. Cells at the late apoptosis stage were significantly increased after treatment for 48 h compared to untreated cells (p-value < 0.01).Totally, the number of intact cancer cells was significantly reduced in EPS-1treated cells relative to untreated ones along the three time points and this reduction was increased most after 72 h of exposure (24 h, p-value < 0.05; 48 h, p-value < 0.05; 72 h p-value < 0.01) indicating the elevation of total percentage of cell deathin response to treatment (Fig 3).

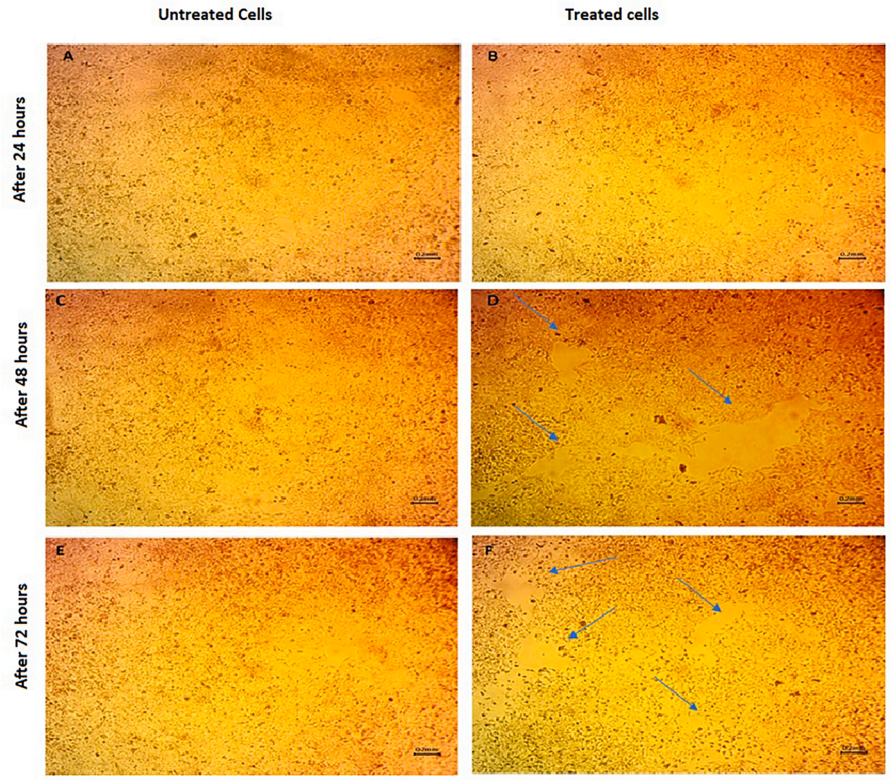


Fig. 1. Microscopical examination of EPS-1 treated and untreated Huh7 cells. (A, C, and D) represent microscopical photos of untreated Huh7 cells after 24, 48, and 72 h respectively. (B, D, and F) represent microscopical photos of EPS-1 treated Huh7 cells after 24, 48, and 72 h respectively. The arrows point to the empty spaces observed in EPS-1 treated culture plates after 48 and 72 h. Scale bar 0.2 mm.

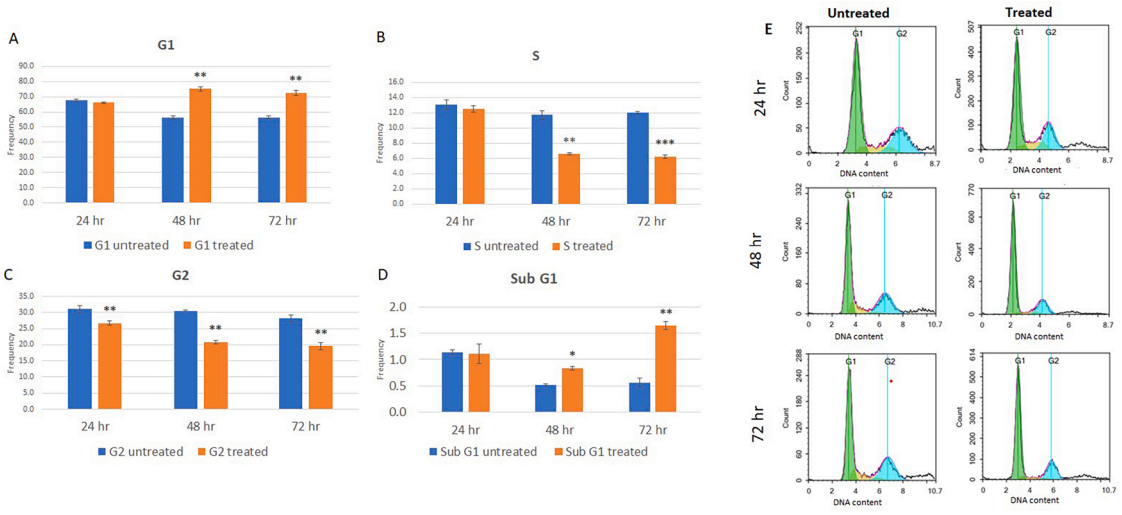


Fig. 2. Cell cycle analysis of EPS-1 treated and untreated control Huh7 cells. (A) A bar chart representing the G1 phase after 24, 48, and 72 h. It shows a significant elevation in the number of treated cells at the G1 phase in comparison to untreated cells after 48 and 72 h.**(B)** A bar chart representing the S phase after 24, 48, and 72 h. **(C)** A bar chart representing the G1 phase after 24, 48, and 72 h. **(D)** A bar chart representing the Sub G1 phase after 24, 48, and 72 h. **(E)**Flow cytometry charts of cell cycle analysis after 24, 48, and 72 h. Data are represented as mean ± SEM, $n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

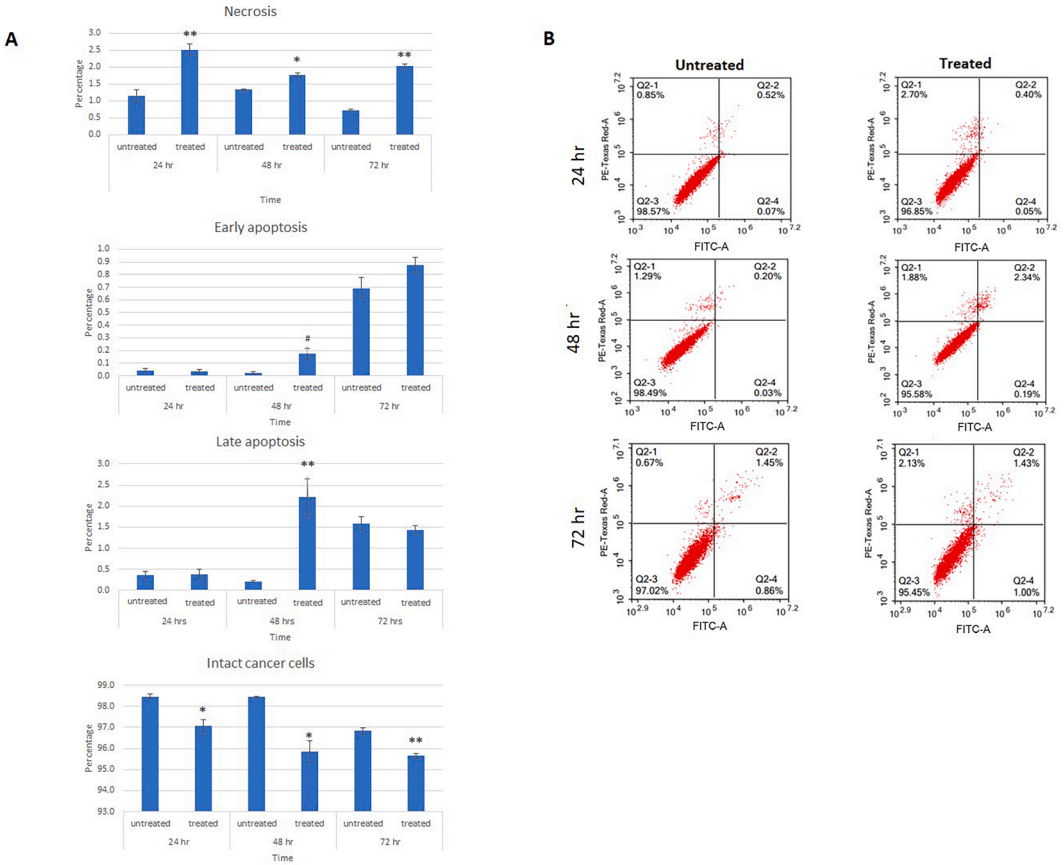


Fig. 3. Apoptosis analysis of EPS-1 treated and untreated Huh7 cells. (A) A bar chart representing the percentage of cells at necrosis, early apoptosis, late apoptosis, and intact cell phase after 24, 48, and 72 h. (B) Apoptosis flow cytometry dot plot of EPS-1 treated and untreated Huh7 cells after 24, 48, and 72 h. Data are represented as mean ± SEM, $n = 3$, $^{\#}p = 0.05$,$^{*}p < 0.05$, $^{**}p < 0.01$.

3.4. Effect of B. sonorensis SAmt2 EPS-1 on Huh7 cell autophagic cell death

We investigated the impact of *B. sonorensis* SAmt2 EPS-1 on the autophagy process within Huh7 cells using flowcytometry. Treating Huh7 cells with *B. sonorensis* SAmt2 EPS-1 for 24, 48, and 72hours showed a significant increase in the autophagic activity in treated cellsafter 48 h (p-value < 0.01) indicating the impact for our compound onautophagic/lysosomal cell death and the optimum time was 48 h (Fig. 4).

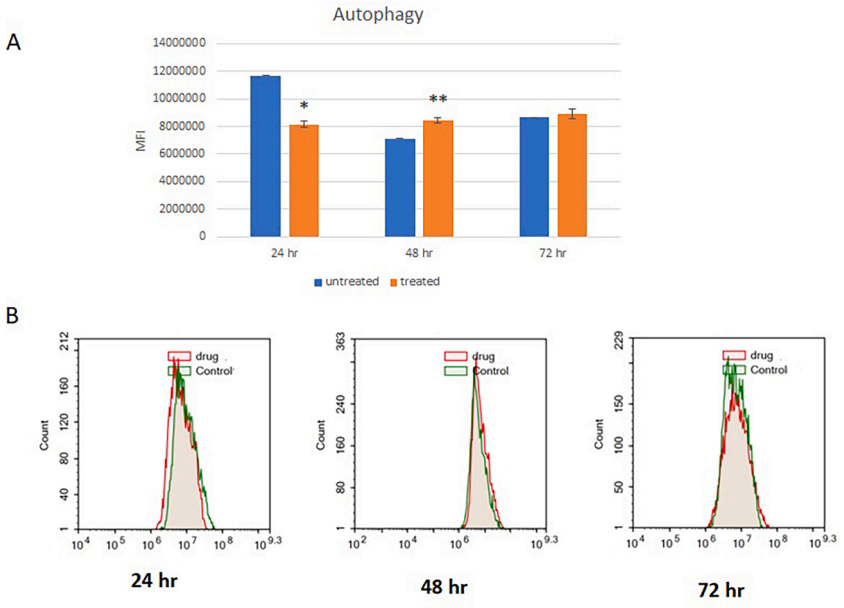


Fig. 4. Autophagy analysis of EPS-1 treated and untreated Huh7 cells. (A) A bar chart representing the mean fluorescence intensity (MFI) of cells after 24, 48, and 72 h. (B) Autophagy flow cytometry charts of EPS-1 treated and untreated Huh7 cells after 24, 48, and 72 h. Data are represented as mean ± SEM, $n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$.

3.5. B. sonorensis SAmt2 EPS-1 affects the secretions of Huh7 cells with a shift towards an anti-cancer behavior

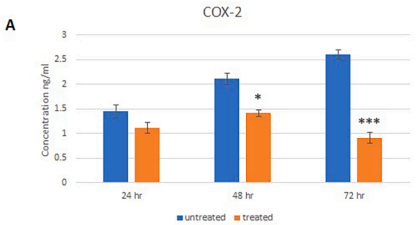
Examining the secretion of COX-2 byEPS-1 treated Huh7 cells revealed alterations in itsprotein's concentration within the conditioned media in comparison to untreated cells. It was observed that COX-2 release was decreased by time, where treatment of cells for 24 h showed slight decline in COX-2 secretion which was more pronounced after 48 h (p-value < 0.05) and was most prominant after 72 h (p-value < 0.001) as shown in Fig 5.

4. Discussion

In our previous work, *B. sonorensis* SAmt2 derived EPS-1 showed promising cytotoxic effects against Huh7 liver cancer cells [18]. Herein, we further investigated the impact of this polymeron cell cycle phase distribution, apoptosis, and autophagy of Huh7 cells. Moreover, we examined whether or not it affects COX-2 secretion in those cells. The results of the current study were in line with our previous work where *B. sonorensis* SAmt2 derived EPS-1 exhibited an antiproliferative activity and enhanced theapoptotic, necrotic, and autophagic cell death.

B. sonorensis was known to be a natural source of exopolysaccharide as mentioned previously [20].This strain exhibited an excellent ability to produce extracellular polysaccharides having various useful bioactive functions especially in medical applications [20]. It was demonstrated that different Bacillus strains provide EPSs with anti-cancer properties such as *Bacillus mycoides* [26], *Bacillus spp*. [27], and *Lactobacillus delbrueckii* Our isolated strain; *B. sonorensis* SAmt2 was capable of producing EPS-1 which showed an anti-cancer activity as demonstrated in our previous work [18] and in the current study.

Cell cycle analysis of treated Huh7 showed asignificant antiproliferative effects for *B. sonorensis* SAmt2 EPS-1 as indicated by increased cell population at the Go/G1 phase (postmitotic gap), after 48 h and 72 h (p-value < 0.01) denoting cell cycle arrest [28]. In addition, our results demonstrated that *B. sonorensis* SAmt2 EPS-1 induced a decline in thepercentage of cells at the Sphase or synthesis phase after 48 h and was more prominent after 72 h (p-value < 0.05 and p-value < 0.01 respectively). At the S phase, DNA is replicated and chromosomal duplication takes place.The proliferative activity of a tumor depends on the proportion of cells in S phase [29]. The decrease in the fraction of cells at the S phase is consistent with G1 arrest indicating inhibited cell division [30–32]. Accordingly, a decreased G2 cell fraction was observed at all time points, since Cells that progress from S phase to G2 are reduced. G2 phase(pre-mitotic phase) is the stageduring which cell growth continues RNA and proteins are synthesized in preparation for the mitosis of the next cycle [33]. Finally, our isolated EPS-1 induced significantelevation in the percentage of pre-G phase populationafter 48 h of treatment. Further exposure for 72 hourssignificantly induced more cell death as manifested by increased pre-G phase cell population (p-value < 0.01).Overall, *B. sonorensis*SAmt2 EPS-1induce cell cycle arrest atthe sub-G1 and G1 phases causing proliferation inhibition and apoptosis. These effects may be attributed to the upregulation of p53



B

Duration	Untreated (ng/ml±SEM)	Treated (ng/ml±SEM)	p-value
24 Hours	1.46±0.12	1.11±0.10	0.105
48 Hours	2.10±0.12	1.41±0.06	0.013
72 Hours	2.60±0.09	0.91±0.10	0.0003

Fig. 5. Quantification of COX-2 concentration in the secretome of Bacillus *sonorensis* SAmt2 EPS-1treated cells vs. untreated Huh7 cells. (A) Protein concentrations COX-2in the conditioned media of treated and untreated Huh7after 24, 48, and 72 h. (B) Tabulation of COX-2 concentrations as quantified by ELISA. Data are represented as mean ± SEM, $n = 3$, $^{*}p < 0.05$, $^{***}p < 0.001$.

expression in the EPS-1 treated Huh7 cells as reported in our previous study [18], since p53 contributes to cell cycle arrest at the sub-G1 and G1 phases enhancing cell death [34].

Cell death is known to be the result of one of two main processes, apoptosis (programmed cell death) and necrosis (uncontrolled cell death), however, several other forms of cell death have been discovered such as autophagy [35].One of the major hallmarks of cancer is resistance to apoptosis, therefore apoptosis induction in cancer cells is an important strategy for cancer treatment. Apoptosis is a biological process by which a cell stops growth and division and enters a phase of controlled death without spillage of its contents. Apoptosis is also known as a gene-directed cell suicide program [35,36].The current study revealed that treating liver cancer cells with *B. sonorensis* SAmt2 EPS-1 induced an elevation in apoptosis as indicated by the significant increase of late apoptotic cell fraction after 48 h exposure (p-value < 0.01). Also, early apoptotic fraction was larger in treated cells relative to non-treated ones after 48 h (p-value $= 0.05$) but with no statistical significance. Such proapoptotic action supports our mentioned results of cell cycle analysis and copes with our previous study [18] as well as Wang et al. 2021 study showed that treatment of Huh7.5 cells with EPS364, which is also a marine bacterial EPS increased both early apoptosis and late apoptosis with a maximin effect after 72 h treatment agreeing with our results [37].

Moreover, our results, showed that *B. sonorensis* SAmt2 EPS-1 induced a significant elevation of necrotic cells at all the three time points; 24, 48 (p-value < 0.05) and 72 h (p-value < 0.01) in a time dependent manner. Necrosis is often the remains of late apoptotic cells, the apoptotic bodies of which have lost integrity (D'Arcy 2019). Collectively, EPS-1 enhanced total cell death where the number of live intact cells significantly decreased after 24 and 48 h of exposure (p-value < 0.01). Further exposure for 72 h induced a more prominent impact (p-value < 0.01).

In addition to apoptosis, programmed cell death via autophagy was also studied, herein. We investigated the effect of *B. sonorensis* SAmt2 EPS-1 on the Huh7 autophagy using flowcytometry analysis technique. Treated cells exhibited an increased autophagic cell death after 48 h of exposure, however, no obvious change in autophagic cell death was observed after 72 h. Autophagy is a highly regulated catabolic degradative process that destroys cytosolic proteins and organelles via lysosomal machinery.Organelles and cytoplasm are engulfed into autophagosomes whichfuse with lysosomes to form autolysosomes to be digested (Chao et al. 2020). Autophagy contributes to cell death through crosstalk with pro-apoptotic pathways [38]. Autophagy was reported to be an inducer of tumor suppression as defects in autophagy are associated with increased tumorigenesis, however, autophagy plays a role of sustaining cellular metabolism and nutrient homeostasis in both normal and tumor cells [39]. Our results agree with the previous findings of Kim et al. (2010) who demonstrated that EPS isolated from probiotic bacteria could activate of autophagic cell death [40].

Collectively, it can be deduced that *B. sonorensis* SAmt2 EPS-1 plays an anticancer role against liver cancer cells via inhibiting cancer cell proliferation and enhancing apoptosis, necrosis, and autophagic cancer cell death. We observed also that mostly the longer exposure duration provides a better impact. Our overall findings support previous reports concerning the antiproliferative and cell death inducing anticancer activities of EPS isolated from various bacterial strains as reviewed lately. One of them is that of Mohd Nadzir et al. (2021). [41]

Cyclooxygenase-2 (COX-2) is released in many types of cancers such as colorectal, pancreatic, breast, as well as liver cancerand secreted by various tumor resident cells [42]. It promotes carcinogenesis enhancing apoptotic resistance, proliferation, angiogenesis, inflammation, invasion, and metastasis. It also plays a key role in cancer cell resistance to chemo- and radiotherapy. Therefore, inhibition of COX-2 is considered an appropriate strategy for cancer therapy [43–45]. In this study, we observed a decline in the release of COX-2 in response to treatment of cells with *B. sonorensis* SAmt2 EPS-1 in a time dependent manner. These results supported our cell proliferation and apoptosis results, since COX-2 inhibits apoptotic cell death and supports cell proliferation. COX-2 specifically inhibits Fas-mediated apoptosis by promoting Mcl-1 expression. In addition, COX-2 contributes to PGE2 production which in turn stimulates EP4 receptors that activate PI3K/Akt signaling enhancing proliferation of cancer cells [43,46,47].Importantly, COX-2 inhibition in preoperative setting may decrease the risk of metastasis in patients and sensitizes cancer cells to radio- and chemotherapy [44]. Therefore, the inhibitory effect of our isolated EPS-1 on COX-2 secretion may be of a great benefit for cancer patients.

5. Conclusion

In conclusion, *B. sonorensis* SAmt2 EPS-1 showed promising effects against liver cancer Huh-7 cells via inducing apoptosis, necrosis and autophagy, in addition to suppressing the release of the apoptosis resistance protein; COX-2. The antiproliferative and cell death enhancing actions of our isolated EPS-1 as well as its inhibitory effect on COX-2 secretion may provide a high possibility to exert therapeutic outcomes in liver cancer. Moreover, the results of the current study confirm the previously reported antitumor potential of *B. sonorensis* SAmt2 EPS-1 illustrated by our previous published work enforcing the proposed anti-oncogenic role of this compound. Finally, the anti-cancer activity displayed by EPS-1 may be of interest for further studies concerning structure-activity relationship as well as future *in vivo* investigations for cancer drug development.

Ethical considerations

Ethical consideration does not apply to this study.

Patient consent for publication

Not applicable.

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CRediT authorship contribution statement

Salma M. Abdelnasser: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nourhan Abu-Shahba:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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