



Activities of the soluble and non-digestible longan (*Dimocarpus longan* Lour.) polysaccharides against HCT-116 cells as affected by a chemical selenylation

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ABSTRACT

The soluble and non-digestible longan (*Dimocarpus longan* Lour.) polysaccharides (LP) with Se content less than 0.01 g/kg were extracted and selenylated chemically with the HNO₃–Na₂SeO₃ system, to prepare two selenylated products namely SeLP1 and SeLP2 with enhanced Se contents of 1.46 and 4.79 g/kg, respectively. LP, SeLP1, and SeLP2 were then measured and compared for their saccharide features and bioactivity in human colon carcinoma HCT-116 cells. Compared with LP, both SeLP1 and SeLP2 contained more neutral saccharides, but showed reduced uronic acid content and undetectable sulfate. Moreover, SeLP1 and especially SeLP2 in the cells showed higher activities than LP, reflected by their enhanced capacity to inhibit cell growth, alter cell morphology, and suppress cell colony formation. Compared with LP, SeLP1 and especially SeLP2 were also more capable of promoting intracellular reactive oxygen species and Ca²⁺ levels, causing mitochondrial membrane potential loss, or inducing cell apoptosis via up- and down-regulating the eight apoptosis-related genes and proteins. Overall, the performed chemical selenylation of LP resulted in obvious changes in these saccharide features and simultaneously enhanced the anti-cancer activity of the selenylated products against the cells clearly, while a higher selenylation extent of the selenylated products consistently caused higher activity towards the cells. The results of this study thus highlighted that this chemical selenylation is applicable when aiming to enhance the bioactivities of natural polysaccharides.

1. Introduction

Colorectal cancer (CRC) is the malignant tumor of the digestive tract with the highest incidence in human. According to the 2020 Global Tumor Burden Report, CRC is one of the three cancers with the new-onset cases in the world, and has the second highest mortality proportion (Global Burden of Disease Cancer Collaboration, 2019). It is worth noting that CRC patients are getting younger, and no longer traditionally believed that cancers only happen after the ages of 50 or 60. A recent study published in JAMA Network Open also pointed out that CRC patients are not only younger, but the younger patients have a lower survival ratio than the patients diagnosed at 51–55 years old (Cheng et al., 2021). In the United States, among adults under 50, CRC is the second most common cancer and the third leading cause of cancer death

(Bhandari et al., 2017; Hofseth et al., 2020). The current treatment modalities for CRC are surgical resection, radiation therapy, and chemotherapy, but these treatments are invariably associated with various side-effects like anemia, immuno-suppression, and alopecia (Khan et al., 2019). Food scientists have paid special attention to the food ingredients with potential functions to promote health or prevent CRC. Natural bioactive substances like dietary fibers, polysaccharides, polyphenols, and other components from various food resources have been investigated for their anti-cancer activities, due to their safety as well as widespread existence in the daily diets (Tuohy et al., 2012; Khan et al., 2019; Wang et al., 2020b).

The *in vivo* and *in vitro* results demonstrated that plant polyphenols could inhibit cancer cell proliferation and induce cell apoptosis or metastasis (Tuohy et al., 2012; Jeyabalan et al., 2014). It was reported

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that the polyphenols bound with foxtail millet bran could inhibit the growth of the human colon cancer HCT-116 cells and induce cell apoptosis *via* activating the signaling pathway mediated by mitochondria and blocking the NF- κ B signaling pathway (Shi et al., 2015), while the epidemiological results revealed that an isoflavone-rich diet might reduce the incidence of hormone-related cancers (Chan et al., 2009). Moreover, adequate intake of the vegetarian foods rich in carotenoids might prevent the non-communicable diseases such as cardiovascular disease, diabetes, and cancers (Dias et al., 2018), while dietary fibers are regarded to have preventive effects on several cancers (Tuohy et al., 2012). Subsequently, mulberry polysaccharides were found to induce cancer cell apoptosis and cell cycle arrest (Chen et al., 2018), while marshmallow root and longan polysaccharides had a direct inhibitory effect on lung cancer (A549) and liver cancer (HepG2) cells (Yang et al., 2013; Hashemifesharaki et al., 2020). Overall, the health benefits of natural bioactive substances such as bioactive polysaccharides have been investigated in both food and medicine fields.

Longan (*Dimocarpus longan* Lour.) belonging to the sapindaceous family is welcome by the consumers (Chen et al., 2021). Longan pulp extracts had several healthy benefits like memory enhancing, anti-cancer, and immuno-modulatory activities (Yang et al., 2011), while the non-digestible longan polysaccharides were considered as the main bioactive components that made a contribution to these health benefits (Zhong et al., 2010). It has been regarded that chemical modification can change the types of chemical groups in the natural polysaccharides, and thus shows an impact on polysaccharide bioactivity including anti-cancer effect (Xie et al., 2020). For example, the sulfated arabinogalactans from *Larix principis-rupprechtii* could induce the apoptosis of A549, HepG2, and breast cancer (MCF-7) cells (Tang et al., 2019), while the acetylated oligosaccharide from *Kappaphycus striatum* had anti-tumor effect on the mice carrying S180 (Yuan et al., 2010). Additionally, the carboxymethylated polysaccharides from *Ganoderma lucidum* were able to inhibit tumor cell proliferation and induce cell cycle arrest (Wang et al., 2009), while the phosphorylated polysaccharides from *Dictyophora indusiata* had obvious inhibition on the growth of MCF-7 and B16 tumor cells (Deng et al., 2015). Polysaccharides also can be conjugated with Se, leading to the formation of Se-containing polysaccharides. As a minor nutrient, Se is important in the body and is well-known for its biological effects on body growth and development (Rayman, 2000). Compared with inorganic Se compounds, organic Se compounds have higher absorption and bioavailability and lower toxicity in the body, especially those in the forms of Se-containing proteins or polysaccharides (Fairweather-Tait et al., 2010; Liu et al., 2018). More importantly, various natural selenylated polysaccharides had been isolated from plants and mushrooms, and were observed to have elevated bioactivity (Shang et al., 2011; Chen et al., 2017). However, Se contents of the selenylated polysaccharides in natural foods usually are very lower; thus, chemical selenylation of natural polysaccharides is an effective way to enhance Se content, accompanying with enhanced bioactivity to various cancer cells. It was confirmed that the selenylated polysaccharides had a higher anti-tumor effect (Wang et al., 2016), anti-oxidation (Liu et al., 2016), and immune regulation (Lee et al., 2018) than the parent polysaccharides or inorganic Se. It was reported the selenylated polysaccharides from *Grifola frondosa* could cause a stronger anti-cancer effect on the tumor-bearing mice by improving the vital immune function (Mao et al., 2016), while those from the *Astragalus membranaceus* root were capable of inhibiting the proliferation of H22 ascites liver cancer and S180 sarcoma cells (Li et al., 2014). Based on these mentioned results, it is likely that an application of chemical selenylation on LP, which is still not clarified so far, might lead to enhanced bioactivity for the resultant selenylated polysaccharides. Thus, such a study deserves our consideration.

In this study, the soluble and non-digestible longan polysaccharides (LP) were extracted, and the two selenylated products (*i.e.* SeLP1 and SeLP2) were thus prepared *via* the chemical selenylation of LP using the $\text{HNO}_3\text{-Na}_2\text{SeO}_3$ system. The saccharide features of LP, SeLP1 and SeLP2

were detected and compared. After then, the bioactivity changes of SeLP1 and SeLP2 in the HCT-116 cells were explored using the unmodified LP as a control. The aim of this study was to reveal whether the chemical selenylation of LP might cause changes in their saccharide features as well as bioactivity against the cells, especially whether the selenylation extent was a factor to govern the bioactivity changes.

2. Materials and methods

2.1. Regents and materials

The dried longan fruits were commercially bought from Maoming market, Guangdong Province, China. The human colon cancer cell line HCT-116 used in this study was purchased from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China).

5-Fluorouracil (5-FU), pectinase (30 kU/g), 3-phenylphenol, and D-galacturonic acid were obtained from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China), while cellulase (50 kU/g), phosphate-buffered saline (PBS), trypsin-EDTA were purchased from Solarbio Science and Technology Co., Ltd. (Beijing, China). Paraformaldehyde was acquired from Meilunbio Institute of Biotechnology (Dalian, China), while McCoy's 5A medium and methyl thiazolyl tetrazolium (MTT) were acquired from Sigma-Aldrich Co. Ltd. (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Wisent Inc. (Montreal, QC, Canada), while dimethyl sulfoxide (DMSO), radio-immunoprecipitation assay (RIPA) lysis buffer, caspase-3 inhibitor Ac-DEVD-CHO, penicillin-streptomycin solution (100X), crystal violet staining solution, ROS assay kit, Annexin V-FITC apoptosis detection kit, Fura-2 pentakis (acetoxymethyl) ester (Fura-2 Am), Hoechst 33258, mitochondrial membrane potential assay kit with JC-1, and BCA protein assay kit were all purchased from Beyotime Institute of Biotechnology (Shanghai, China). The RNAPrep Pure Cell Bacteria kit was purchased from Tiangen Biotech Co. Ltd. (Beijing, China), while NovoScript® Two-Step RT-PCR kit and SYBR qPCR SuperMix Plus were purchased from Novoprotein Biotech, Co., Ltd. (Suzhou, China). All chemicals used were of analytical grade, while water used was generated from Milli-Q Plus system (Millipore Corp., New York, NY, USA).

The primary antibodies β -actin (bs-0061R) was provided by Bioss Biotechnology Co. Ltd. (Beijing, China), while other primary antibodies cytochrome *c* (11940S), Bax (5023S), caspase-3 (9662S), caspase-8 (9746S), caspase-9 (9508S), CHOP (2895S), Bcl-2 (15071S), and DR5 (69400S) were provided by Cell Signaling Technology (Shanghai) Biological Reagents Co., Ltd. (Shanghai, China), while the horseradish peroxidase-labeled goat anti-rabbit IgG (ZB-2301) and goat anti-mouse IgG (ZB-2305) of secondary antibodies were provided by Zhongshan Golden Bridge Bio-technology (Beijing, China).

2.2. Extraction and chemical selenylation of LP

The dried longan was soaked in 80% ethanol solution at 25 °C for 24 h to remove the pigments and soluble monosaccharides or oligosaccharides, mashed into fine particles, mixed with distilled water at the set solid-liquid ratio of 1:15 (w/v) as previously described (Huang et al., 2019), adjusted to pH 4.5 by 1.0 mol/L HCl, added with respective pectinase and cellulase at 150 and 300 U per gram dried longan as a reported study did (Gao et al., 2017), and kept at 50 °C for 4 h with gentle stirring. The separated supernatant *via* a centrifugation at 8000 \times g for 10 min was then concentrated into one-twentieth volume using a vacuum rotary evaporator (Yarong, Shanghai, China) at 50 °C, and precipitated by anhydrous ethanol of three volumes at 4 °C for 24 h. The collected precipitates were washed with anhydrous ethanol for three times, re-dissolved in water, and lyophilized to obtain LP.

LP were selenylated using the $\text{HNO}_3\text{-Na}_2\text{SeO}_3$ method as previously described (Cheng et al., 2018). In brief, LP of 500 mg were mixed with 0.5% HNO_3 solution of 10 mL, added with Na_2SeO_3 of 25 or 75 mg, and kept at 75 °C for 8 h with a gentle stirring. After the reaction, the whole

reaction system was cooled and centrifuged at $8000\times g$ for 10 min. The separated supernatant was precipitated with anhydrous ethanol of three volumes at $4\text{ }^{\circ}\text{C}$ for 24 h. The collected precipitates were washed with anhydrous ethanol for three times, dissolved in water, and then lyophilized to yield two selenylated polysaccharide products namely SeLP1 (using 25 mg Na_2SeO_3) and SeLP2 (using 75 mg Na_2SeO_3). Moreover, LP and Na_2SeO_3 of 75 mg were mixed with 10 mL water and subjected to the same reaction and treatments, while the obtained LP were used as a control in this study.

2.3. Chemical and FT-IR assays

Content of neutral saccharides was assayed as previously described (DuBois et al., 1956), while that of uronic acid was determined by the meta-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1962). Sulfate content was assessed by a turbidimetric detection (Dodgson and Price, 1962), while selenium content was detected as previously described (Mohd-Taufek et al., 2016) using Agilent 7800 ICP-MS Inductively Coupled Plasma Mass Spectrometry (Agilent Technologies, Santa Clara, CA, USA).

The FT-IR spectra of the samples were recorded with a Nicolet iS50 FT-IR Spectrometer (ThermoFisher Scientific, Karlsruhe, Germany), using the KBr pellets and wavenumbers ranging from 400 to 4000 cm^{-1} .

2.4. Cell culture and assay of growth inhibition

As recommended, the HCT-116 cells were cultured in a humidified incubator at $37\text{ }^{\circ}\text{C}$ with 5% CO_2 using the complete medium McCoy's 5A medium fortified with 10% FBS, 100 U/mL penicillin/streptomycin, and 2.2 g/L NaHCO_3 .

Cell viability was measured by an MTT assay. In brief, the cells of 100 μL were seeded in 96-well plates (1×10^5 cells/well) for 24 h to allow cell attachment. After discarding the medium, the cells were treated with the complete medium of 100 μL (negative control), 5-FU of 100 $\mu\text{mol/L}$ (positive control), Na_2SeO_3 of 1–16 $\mu\text{g/mL}$ (inorganic Se control), or the three polysaccharide samples (LP, SeLP1 and SeLP2) at dose levels of 50–800 $\mu\text{g/mL}$ for 24 and 48 h, respectively. After medium removal and PBS (10 mmol/L, pH 7.0) washing, 100 μL MTT solution (10 μL 5 mg/mL MTT in 90 μL medium) was added to each well, while the cells were incubated at $37\text{ }^{\circ}\text{C}$ for another 4 h. After a supernatant removal, 150 μL DMSO was added to each well, while the optical density of each well at 490 nm was determined using a microplate reader (Bio Rad Laboratories, Hercules, CA, USA). Growth inhibition of the assessed samples on the cells were calculated as previously described (Mao et al., 2016), while the cells of the negative control were regarded without any growth inhibition.

2.5. Hoechst 33258 staining

A fluorescence probe Hoechst 33258 was used for nuclear staining to observe cell morphology. In brief, the cells of 2 mL were seeded in 6-well plates (1×10^5 cells/well) for 24 h to allow cell attachment, and then treated by the complete medium, LP (800 $\mu\text{g/mL}$), SeLP1 (400 and 800 $\mu\text{g/mL}$), and SeLP2 (400 and 800 $\mu\text{g/mL}$) for 24 and 48 h, respectively. After discarding the medium, the cells were washed with the PBS twice, fixed with 4% methanol of 0.5 mL at $4\text{ }^{\circ}\text{C}$ for 10 min, washed with the PBS thrice, stained with Hoechst 33258 of 1 mL at $25\text{ }^{\circ}\text{C}$ for 5 min in the dark, and then observed under a fluorescence microscope (ThermoFisher Scientific, Invitrogen EVOS FL Auto 2, Carlsbad, CA, USA) using an objective of 20-folds and excitation/emission wavelengths of 350/460 nm, respectively.

2.6. Assay of cell colony formation

The cells of 2 mL were seeded in 6-well plates (1×10^3 cells/well) for 24 h to allow cell attachment. After then, the cells were treated with the

complete medium, LP (800 $\mu\text{g/mL}$), SeLP1 (400 and 800 $\mu\text{g/mL}$), and SeLP2 (400 and 800 $\mu\text{g/mL}$) for 48 h. After medium removal, the medium with 5% FBS was added every 3 d till the cells were cultured for 14 and 21 d. After discarding the medium, the cells were washed with the PBS thrice, fixed with 4% paraformaldehyde of 1 mL at $4\text{ }^{\circ}\text{C}$ for 15 min, washed with the PBS thrice, stained with crystal violet dye of 1 mL at $25\text{ }^{\circ}\text{C}$ for 10 min in the dark, washed with the PBS thrice, and then photographed after cell drying.

2.7. Analysis of mitochondrial membrane potential, intracellular ROS and Ca^{2+}

The cells of 2 mL were seeded into 6-well plates (1×10^5 cells/well) for 24 h to allow cell attachment, and then treated by the complete medium, LP (800 $\mu\text{g/mL}$), SeLP1 (400 and 800 $\mu\text{g/mL}$), and SeLP2 (400 and 800 $\mu\text{g/mL}$) for 24 and 48 h, respectively.

To assay mitochondrial membrane potential (MMP), the cells were collected and washed with PBS twice, while the JC-1 dye staining solution of 1 mL was added to each well at $37\text{ }^{\circ}\text{C}$ for 20 min in the dark. The cells were seeded into 96-well plates to measure fluorescence intensity using the fluorescence microplate reader and fixed excitation/emission wavelengths of 525/590 nm. MMP of the treated cells was expressed as the red/green fluorescent ratio as previously described (Wang and Zhao, 2017).

To assay the intracellular ROS, the cells were also washed with PBS twice, while the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) staining solution (5 $\mu\text{mol/L}$) of 1 mL was added to each well at $37\text{ }^{\circ}\text{C}$ for 20 min in the dark. The cells were seeded into 96-well plates to detect fluorescence intensity using the fluorescence microplate reader and excitation/emission wavelengths of 488/525 nm. Relative intracellular ROS levels of the treated cells were expressed as the percentages of the control cells.

To assay the intracellular Ca^{2+} , the cells were washed twice with Krebs-Ringer buffer (pH 7.4), while Fura-2 AM staining solution (5 $\mu\text{mol/L}$) of 1 mL was added to each well at $37\text{ }^{\circ}\text{C}$ for 40 min in the dark. The cells were seeded into 96-well plates to measure fluorescence intensity using the fluorescence microplate reader and respective excitation and emission wavelengths of 340/380 and 510 nm. Intracellular Ca^{2+} (i.e. $[\text{Ca}^{2+}]_i$) was calculated as described previously (He et al., 2019).

2.8. Assay of cell apoptosis by flow cytometry

The cells of 2 mL were inoculated at 6-well plates (1×10^5 cells/well) for 24 h to allow cell attachment, and then treated by the complete medium, LP (800 $\mu\text{g/mL}$), SeLP1 (400 and 800 $\mu\text{g/mL}$), and SeLP2 (400 and 800 $\mu\text{g/mL}$) for 24 and 48 h. The cells were collected and washed with PBS twice, while Annexin V-FITC binding buffer of 195 μL , Annexin V-FITC of 5 μL , and PI staining solution of 10 μL were added to each well at $25\text{ }^{\circ}\text{C}$ for 10 min in the dark. The cells were analyzed using the flow cytometer (Type BDFACS Aria II, BD Bioscience, Franklin Lakes, NJ, USA) to obtain the respective percentages of viable, necrotic, early apoptotic, and late apoptotic cells (i.e. Q1–Q4).

2.9. Reverse transcription quantitative real-time PCR assay

The cells of 2 mL were inoculated at 6-well plates (1×10^5 cells/well) for 24 h to allow cell attachment, and then treated by the complete medium, LP (800 $\mu\text{g/mL}$), SeLP1 (400 and 800 $\mu\text{g/mL}$), and SeLP2 (400 and 800 $\mu\text{g/mL}$) for 48 h. The preformed reverse transcription quantitative real-time PCR (RT-qPCR) assay was divided into three brief steps: (1) total RNA was extracted using the RNAprep pure cell kit; (2) reverse transcription of the RNA into complementary DNA (cDNA) using the NovoScript® two-step RT-PCR kit; and (3) amplification of the cDNA using the NovoScript® SYBR qPCR SuperMix Plus and Biosystems StepOnePlus real-time PCR system (Life Technologies Corp., Carlsbad, CA,

USA). The relative expression levels of the target genes were calculated using the classic $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The β -actin housekeeping gene was used as the internal standard. The primers were designed with the sequences listed in Table 1.

2.10. Assay of western-blotting

The cells of 5 mL were inoculated at a 25 cm² cell culture flask (1×10^6 cells/flask) for 24 h, treated by the complete medium, LP (800 μ g/mL), SeLP1 (400 μ g/mL), SeLP2 (400 μ g/mL), Ac-DEVD-CHO (50 μ mol/L), and SeLP2 (400 μ g/mL) plus Ac-DEVD-CHO (50 μ mol/L) for 48 h, washed with PBS twice, added with radio-immunoprecipitation assay (RIPA) lysis buffer of 100 μ L containing PMSF (1 mmol/L), lysed for 30 min at 4 °C, and centrifuged at 12,000 \times g for 5 min. The collected supernatants were detected for total protein concentrations using the BCA protein analysis kit. An equal amount of the protein (20 μ g) was separated on 12% SDS-PAGE gel and electro-transferred to the nitrocellulose membranes. The membranes were blocked with 5% skimmed milk (dissolved in the TBST buffer) for 2 h at 37 °C, and incubated with the primary antibodies (1:1000 dilution) at 4 °C for 12 h. Afterwards, the membranes were washed three times with the TBST buffer, incubated with the horseradish peroxidase-conjugated secondary antibodies (1:5000 dilution) at 37 °C for 1 h, and re-washed three times with TBST buffer. An enhanced chemiluminescence reagent (Biosharp, Beijing, China) of 100 μ L was used to cover the bands. An Image Quant LAS 500 (Fujifilm, Tokyo, Japan) was applied to detect the signals of protein bands, while the images of the blots were also captured. The quantification of the target protein bands was performed using the ImageJ 2x software (National Institutes of Health, Bethesda, MD, USA). Moreover, β -actin was used as an endogenous standard to normalize band density.

2.11. Statistical analyses

All reported values were expressed as the means \pm standard deviations from three independent experiments or assays. Statistical Program for Social Sciences 16.0 software package (SPSS Inc., Chicago, IL, USA) and Duncan's multiple range tests were used to analyze significant differences ($p < 0.05$) between the mean values of the individual group.

3. Results

3.1. Chemical and FT-IR features of the polysaccharide samples

The measured contents of neutral saccharides, uronic acid, sulfate,

Table 1
Primer sequences used in real-time PCR assays.

Gene	Species	Primer
Bax	Human	Forward 5'-CCAGAGCGGGGATGATT-3'
		Reverse 5'-CTGATCAGTTCGGCACCTT-3'
Bcl-2	Human	Forward 5'-CTTTGAGTTCGGTGGGGTCA-3'
		Reverse 5'-GGGCCGTACAGTTCACAAA-3'
Caspase-3	Human	Forward 5'-TTGAGACAGACAGTGGTGTGATGATG-3'
		Reverse 5'-ATAATAACCAAGTGCTGTGGAGTATGC-3'
Caspase-8	Human	Forward 5'-CAAACCTCACAGCATTAGGGAC-3'
		Reverse 5'-ATGTTACTGTGGTCCATGAGTT-3'
Caspase-9	Human	Forward 5'-CTGCTGCGTGGTGGTCAATTTC-3'
		Reverse 5'-CACAATCTTCTCGACCGACACAGG-3'
CHOP	Human	Forward 5'-TAAAGATGAGCGGGTGGCAG-3'
		Reverse 5'-CTGCCATCTCTGCAGTTGGA-3'
Cytochrome c	Human	Forward 5'-GAGTAATAATTGGCCACTGCCT-3'
		Reverse 5'-AATCAGGACTGCCCAACAAA-3'
DR5	Human	Forward 5'-CTGATCACCCAACAAGACCTAG-3'
		Reverse 5'-GATGCAATCTTACCGTCTTCT-3'
β -Actin	Human	Forward 5'-CCACCATGTACCCTGGCAT-3'
		Reverse 5'-ACTCCTGCTGTGATCCAC-3'

and Se of LP, SeLP1 and SeLP2 are summarized in Table 2. The neutral saccharide content of LP was 753.9 g/kg, which was lower than these contents of SeLP1 (783.8 g/kg) and SeLP2 (792.1 g/kg). Meanwhile, the uronic acid content of LP was 117.8 g/kg, which was higher than these contents of SeLP1 (80.7 g/kg) and SeLP2 (78.1 g/kg). LP was measured with a sulfate content of 17.7 g/kg. However, SeLP1 and SeLP2 were detected without sulfate because they showed a negative response to the applied reagents. In addition, Se content of LP was less than 0.01 g/kg, while SeLP1 and SeLP2 had much enhanced Se contents of 1.46 and 4.79 g/kg, respectively. These data indicated that the conducted chemical selenylation of LP using the HNO₃-Na₂SeO₃ system could incorporate Se element to the final selenylated products SeLP1 and SeLP2 efficiently, and also led to the total or partial removal of sulfate or uronic acid. Furthermore, compared with SeLP1, SeLP2 also had much reduction in uronic acid content but more increases in neutral saccharide and Se contents. Based on these results, it could be speculated that LP, SeLP1 and SeLP2 might have different activities to the cells, because the three polysaccharide samples had different values in these measured chemical indices.

The obtained FT-IR spectrum of LP, SeLP1 and SeLP2 are shown in Fig. 1. Briefly, SeLP1 and SeLP2 showed higher absorption than LP at the two peaks around 1080 and 620 cm⁻¹, which are regarded as typical peaks of respective Se=O stretching vibration and C-O-Se bending vibration (Chen et al., 2016). This fact suggested that SeLP1 and SeLP2 had higher Se contents than LP, confirming again that Se element was bound to LP molecules during the chemical selenylation. Meanwhile, LP showed two absorption peaks around 1270 and 820 cm⁻¹, which are contributed by the stretching vibration of S=O and C-O-S (Wang et al., 2020a). However, SeLP1 and SeLP2 were observed to have a total disappearance for the peaks around 1270 and 820 cm⁻¹, reflecting their decreased sulfate contents. Overall, the FT-IR assay results showed a conclusion consistence with the two results of the chemical analyses (Table 2); that is, Se incorporation and sulfate removal of SeLP1 and SeLP2.

3.2. Cytotoxic effects of the polysaccharide samples on the cells

Cytotoxic effects of the three polysaccharide samples (LP, SeLP1 and SeLP2), Na₂SeO₃, and positive 5-FU on HCT-116 cells were assessed at the targeted dose levels (Fig. 2). 5-FU as a classic chemotherapeutic agent at 100 μ mol/L showed clear cytotoxicity (or growth inhibition) on the cells, causing the inhibition values of 43.5% (24 h) and 68.7% (48 h). Na₂SeO₃ at lower dose levels (1–8 μ g/mL) had obvious cytotoxicity on the cells, resulting in the inhibition values of 8–84%; moreover, Na₂SeO₃ at 16 μ g/mL together with 48 h treatment time even caused efficient cell death (inhibition value of 94%) (Fig. 2A). This fact meant that Na₂SeO₃ was very toxic to the cells. The obtained data (Fig. 2B and C) also indicated that LP, SeLP1 and SeLP2 also had cytotoxic functions to the cells, and dose- and time-dependently caused growth suppression on the cells. In detail, LP at 50–800 μ g/mL led to inhibition values of 6.1–13.5% (24 h) and 8.7–15.4% (48 h), SeLP1 at these dose levels resulted in inhibition values of 14.2–41.2% (24 h) and 18.1–71.8% (48 h), while SeLP2 at the same dose levels yielded inhibition values of 18.5–50.5% (24 h) and 21.3–84.9% (48 h). It was not possible to calculate the IC₅₀ value of LP scientifically, but SeLP1 and SeLP2 could be estimated with

Table 2
Some chemical indices (g/kg) of the prepared longan polysaccharides (LP) and two selenylated polysaccharides (SeLP1 and SeLP2).

Sample	Neutral saccharide	Uronic acid	Sulfate	Se
LP	753.9 \pm 9.7 ^b	117.8 \pm 7.8 ^a	17.7 \pm 4.1	Less than 0.01
SeLP1	783.8 \pm 11.4 ^a	80.7 \pm 5.1 ^b	Not detectable	1.46 \pm 0.08 ^b
SeLP2	792.1 \pm 10.1 ^a	78.1 \pm 4.8 ^b	Not detectable	4.79 \pm 0.13 ^a

Different lowercase letters after the values as the superscripts at the same column indicate significant differences of the mean values ($P < 0.05$).

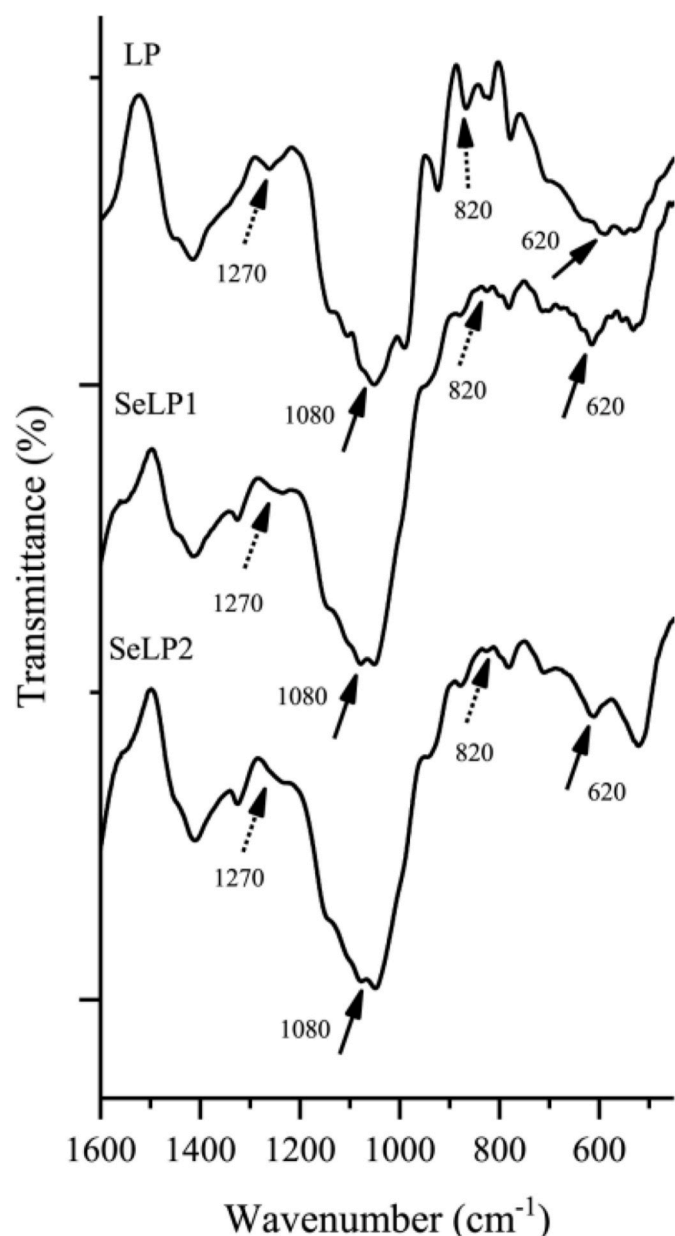


Fig. 1. FT-IR spectra of the longan polysaccharides (LP) and selenated polysaccharide products (SeLP1 and SeLP2).

respective IC_{50} values of 1330 and 640 (24 h) or 360 and 180 $\mu\text{g}/\text{mL}$ (48 h). The results confirmed that SeLP1 and especially SeLP2 had higher growth inhibition than LP on the cells, suggesting the used chemical selenylation as well as higher selenylation extent partly made a contribution to the enhanced growth inhibition of SeLP1 and SeLP2 on the cells. In other words, the performed chemical selenylation of LP led to higher activity in the cells, and a higher selenylation extent also caused an activity enhancement.

When the cells were exposed to LP, SeLP1 and SeLP2 for 24 and 48 h, Hoechst 33258 staining results (Fig. 3) demonstrated that the treated cells had morphological changes, such as the decreased cell density in the observation vision, nuclei shrinkage and fragmentation, and formation of apoptotic bodies. In general, longer treatment time induced more pronounced morphological changes, while SeLP1 and especially SeLP2 had a higher capacity than LP to alter cell morphology. Furthermore, when the cells were exposed to LP, SeLP1 and SeLP2 for 14 and 21 d to reflect the long-term growth inhibition of the three samples, the results (Fig. 4) also indicated that SeLP1 and especially SeLP2 had

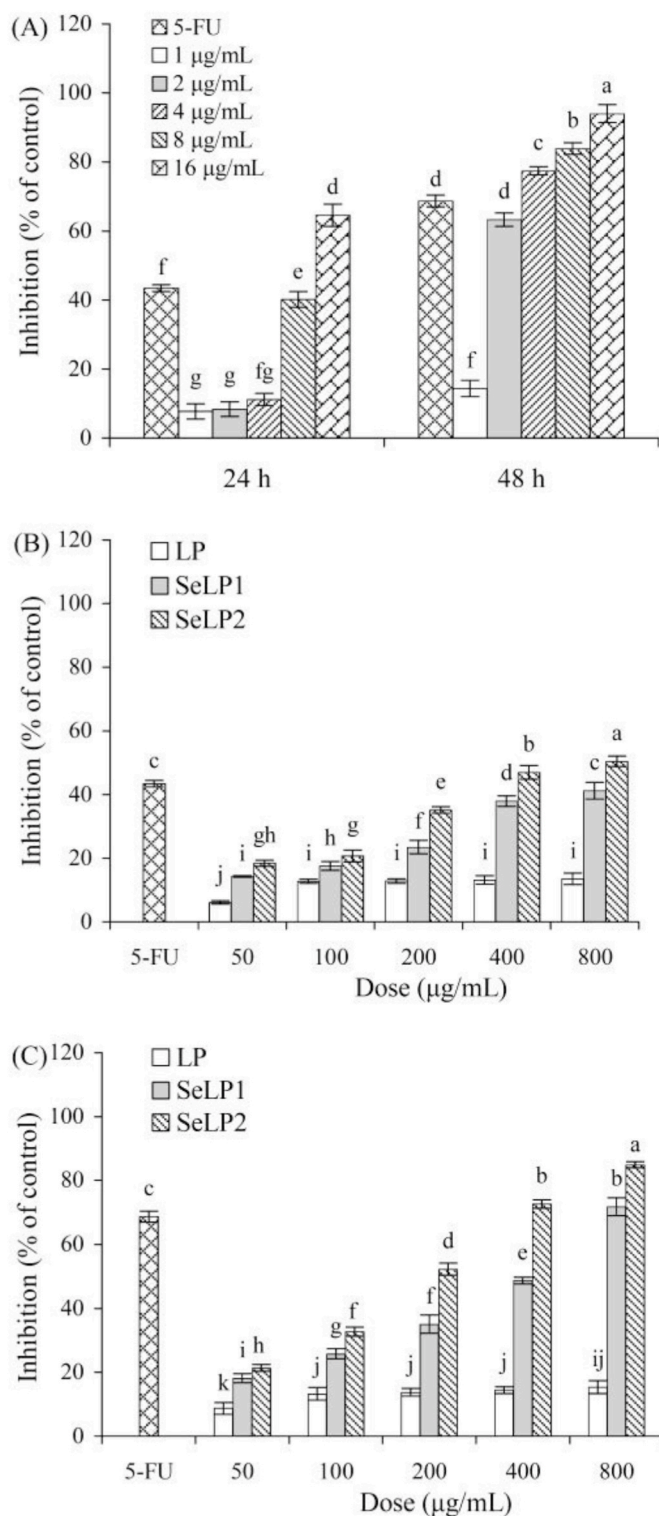


Fig. 2. Growth inhibition of Na_2SeO_3 , longan polysaccharides (LP) and selenated polysaccharides (SeLP1 and SeLP2) on HCT-116 cells with the treatment times of 24 h and 48 h. Different lowercase letters above the columns indicate significant differences of the mean values ($p < 0.05$).

powerful ability than LP to inhibit cell colony formation. Overall, the results from the two assays consistently revealed that the chemical selenylation of LP led to higher inhibitory activity to the cells, and higher selenylation extent caused an activity enhancement.

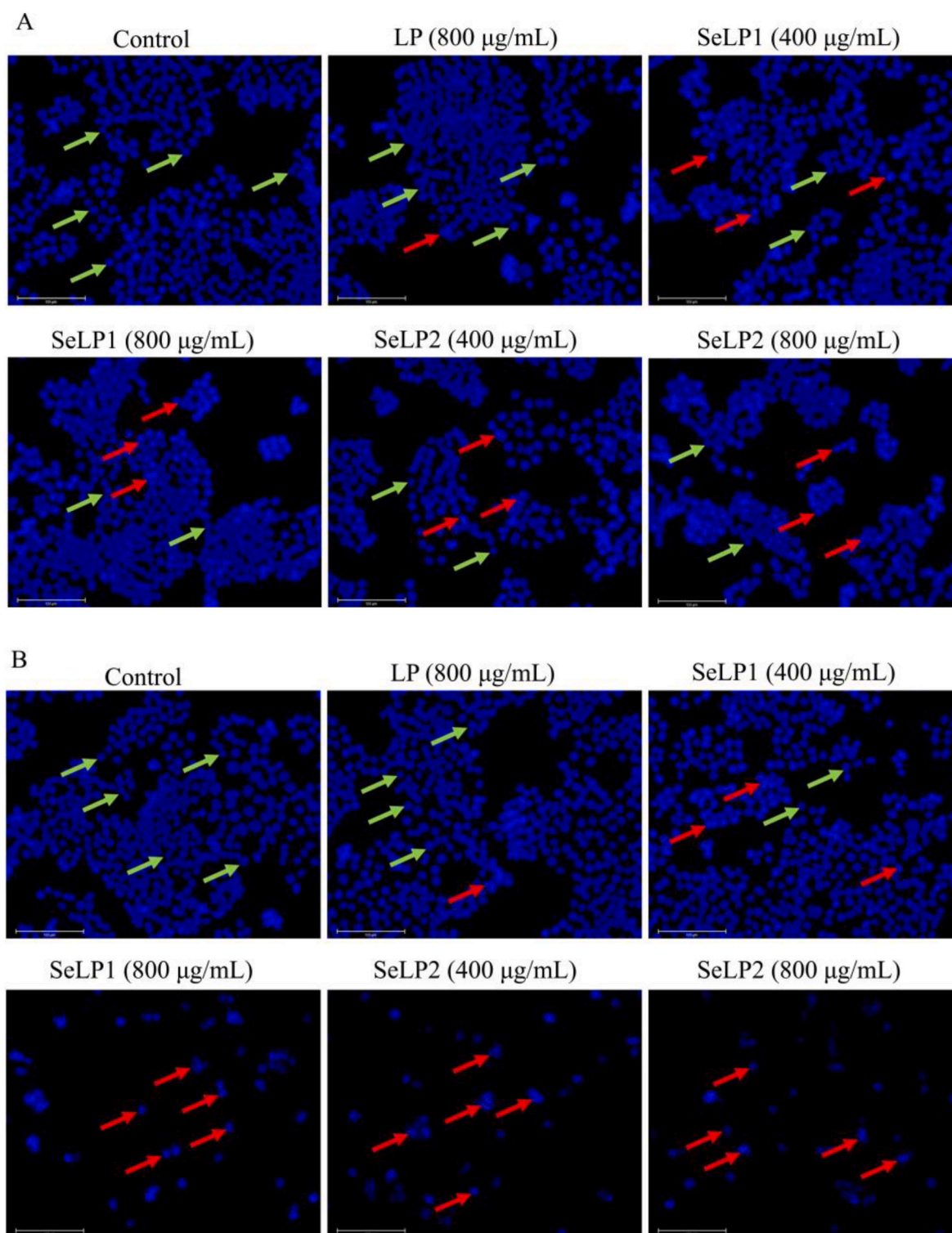


Fig. 3. Morphological features of HCT-116 cells treated with or without longan polysaccharides (LP) and selenylation polysaccharide products (SeLP1 and SeLP2) for 24 h (A) and 48 h (B).

3.3. Intracellular ROS, Ca^{2+} and MMP loss in response to the polysaccharide samples

When the cells were exposed to LP, SeLP1, and SeLP2 for 24 and 48 h, the results (Fig. 5A) showed that the treated cells dose- and time-dependently showed increased ROS levels, indicating the three polysaccharide samples had a pro-oxidation in the cells. In detail, compared with the control cells, the LP-treated cells using a dose level of 800 µg/

mL had ROS levels of 112.7% (24 h) and 119.6% (48 h), while the SeLP1-treated cells using dose levels of 400 and 800 µg/mL showed ROS levels of 142.6–148.8% (24 h) and 163.9–170.7% (48 h). Additionally, the SeLP2-treated cells using dose levels of 400 and 800 µg/mL possessed ROS levels of 163.9–170.7% (24 h) and 287.7–322.9% (48 h). Clearly, SeLP1 and especially SeLP2 were more active than LP to promote ROS generation in the cells.

Furthermore, the treated cells also showed decreased MMP, reflected

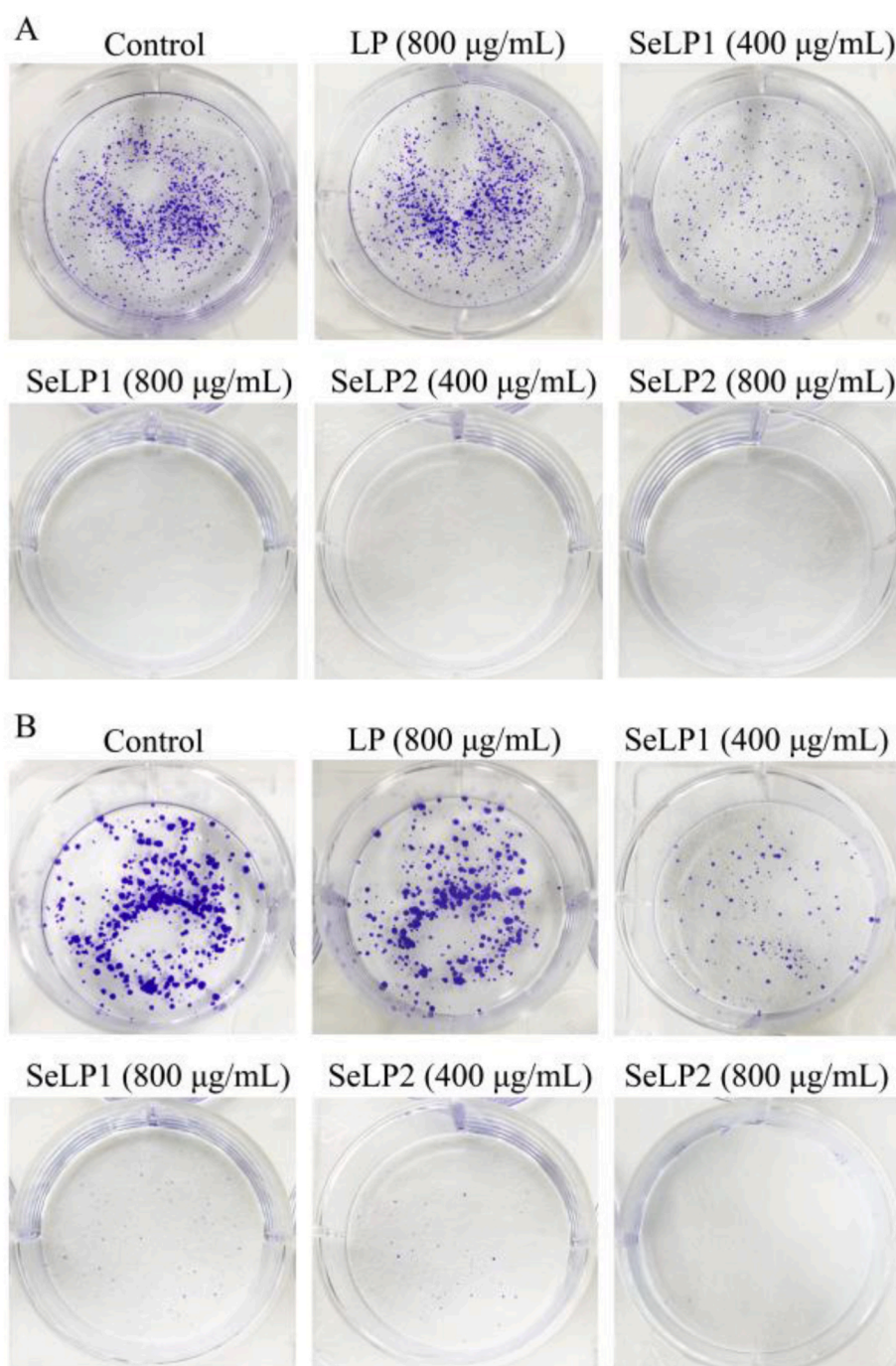


Fig. 4. The observed long-term anti-proliferation of longan polysaccharides (LP) and selenylated polysaccharide products (SeLP1 and SeLP2) on the HCT-116 cells with the exposure times of 14 (A) and 21 d (B).

by their reduced ratios of red/green fluorescence (Fig. 5B). The control cells had the ratio of 14.1 (24 h) or 17.9 (48 h), while the LP-treated cells using a dose level of 800 µg/mL showed lower ratios of 11.1 (24 h) or 13.2 (48 h). Additionally, the SeLP1-treated cells using dose levels of 400 and 800 µg/mL had the reduced ratios of 5.8–6.2 (24 h) or 2.1–5.8 (48 h), while the SeLP2-treated ones using the same dose levels showed the much reduced ratios of 4.5–5.3 (24 h) or 1.4–2.1 (48 h). These data suggested that LP, SeLP1, and SeLP2 were able to damage mitochondrial membranes of the cells, resulting in the MMP loss. Data comparison also revealed that SeLP1 and especially SeLP2 were more potent than LP to cause MMP loss.

The treated cells also had substantial intracellular Ca^{2+} release, reflected by the increased values of intracellular Ca^{2+} (Fig. 5C). Compared

with the control cells (100% $[\text{Ca}^{2+}]_i$ value), the cells treated by LP at 800 µg/mL showed $[\text{Ca}^{2+}]_i$ values of 105.5% (24 h) or 106.3% (48 h), while those treated by SeLP1 at 400 and 800 µg/mL gave $[\text{Ca}^{2+}]_i$ values of 110.9–113.3% (24 h) or 125.9–160.6% (48 h). Furthermore, the cells treated by SeLP2 at 400 and 800 µg/mL showed much increased $[\text{Ca}^{2+}]_i$ values of 120.6–125.4% (24 h) or 161.3–173.8% (48 h). Overall, LP and SeLP2 had the lowest and highest activities in the cells to cause intracellular Ca^{2+} release, confirming again that the used chemical selenylation of LP endowed SeLP1 and SeLP2 with higher activities in the cells to promote intracellular Ca^{2+} release, while higher selenylation extent was accompanied with activity enhancement. In general, a higher but unusual level of ROS in the cells can induce endoplasmic reticulum (ER) stress; subsequently, ER stress leads to the release of large amounts of

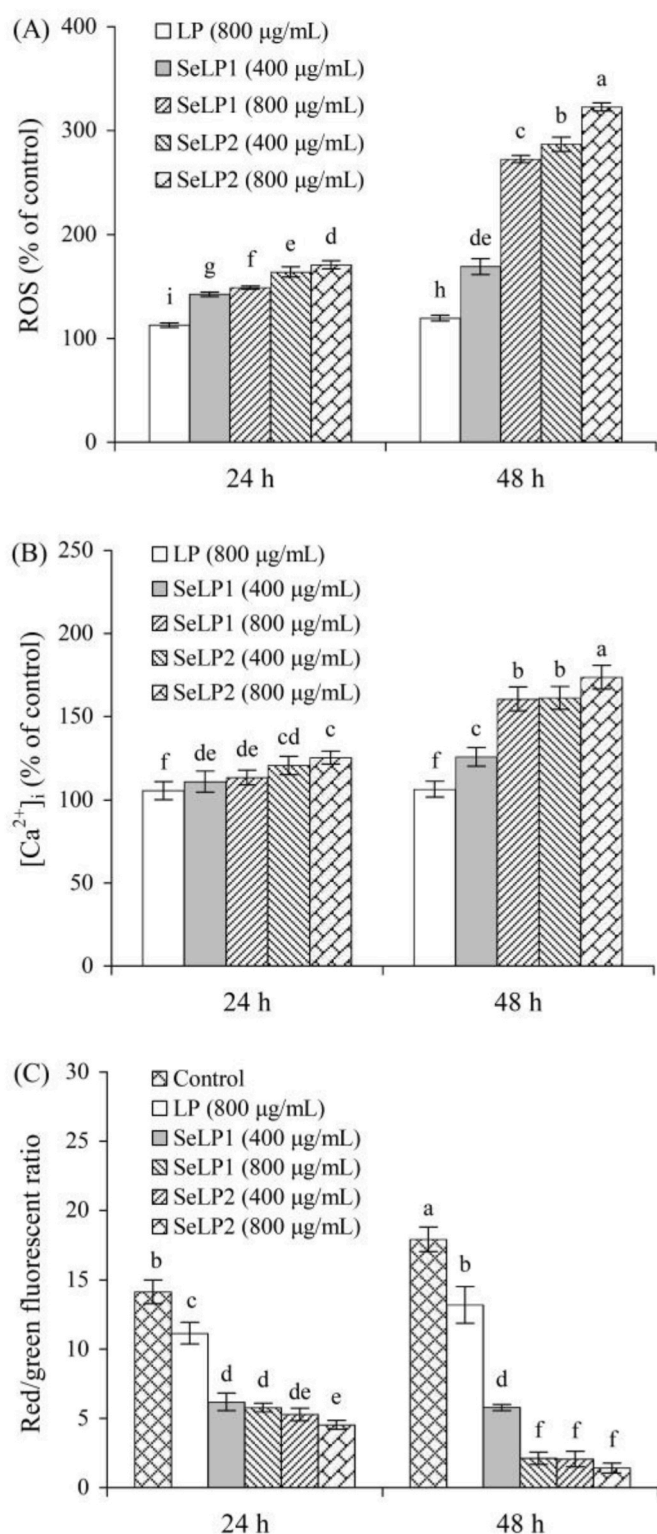


Fig. 5. The measured ROS formation (A), mitochondrial membrane potential loss (B), and intracellular Ca²⁺ (C) of the HCT-116 cells treated with or without longan polysaccharides (LP) and selenylation polysaccharide products (SeLP1 and SeLP2) for 24 and 48 h. Different lowercase letters above the columns indicate significant differences of the mean values ($p < 0.05$).

Ca²⁺ into the cytoplasm, while Ca²⁺ overload finally causes MMP loss (Wang and Zhao, 2017). Based on these obtained results (Fig. 5), it was possible that LP, SeLP1, and SeLP2 might exert their activities to the cells via inducing cell apoptosis, and the performed chemical

selenylation endowed SeLP1 and SeLP2 with increased apoptosis induction.

3.4. Apoptosis induction of the polysaccharide samples to the cells

The cells were thus exposed to LP, SeLP1 and SeLP2 at the targeted dose levels and treatment times. The apoptotic proportions (Q2 + Q4, early plus late apoptotic cells) of the treated cells were measured (Fig. 6). The control cells only had the apoptotic proportions of 7.0% (24 h) or 7.3% (48 h), while the LP-treated cells using a dose level of 800 µg/mL had the apoptotic proportions of 8.7% (24 h) or 10.0% (48 h). Meanwhile, the cells treated by SeLP1 at 400 and 800 µg/mL had the apoptotic proportions of 18.5–20.0% (24 h) or 39.9–56.9% (48 h), while these treated by SeLP2 at 400 and 800 µg/mL showed the apoptotic proportions of 22.1–29.8% (24 h) or 57.3–66.1% (48 h). That is, SeLP1 and especially SeLP2 had higher apoptosis induction than LP, suggesting the used chemical selenylation as well as higher selenylation extent make a contribution to the enhanced apoptosis induction of SeLP1 and SeLP2.

3.5. Expression changes of the apoptosis-related genes and proteins in the treated cells

When the cells were exposed to LP, SeLP1, and SeLP2, their mRNA expression levels of 8 apoptosis-related genes were assayed (Table 3). As usual, the control cells were defined with a relative mRNA expression level of 1.0-fold for these genes. Overall, LP, SeLP1, and SeLP2 in the cells were regarded to up-regulate the expression of the 7 pro-apoptotic genes Bax (1.1–2.6 folds), caspase-3 (1.0–1.4 folds), caspase-8 (1.1–1.5 folds), caspase-9 (1.1–1.9 folds), CHOP (1.0–1.6 folds), cytochrome c (1.0–1.5 folds), and DR5 (1.0–2.0 folds), but down-regulate the expression of an anti-apoptotic gene Bcl-2 (0.7–1.0 folds). Meanwhile, SeLP1 and especially SeLP2 were more active than LP to regulate these gene targets, revealing that the used chemical selenylation as well as higher selenylation extent endowed SeLP1 and SeLP2 with greater apoptosis induction in the cells.

The western-blotting assays were thus conducted to determine the expression changes of the 8 apoptosis-related proteins (Fig. 7). The results (Table 4) demonstrated that SeLP1 and SeLP2 totally showed a higher ability than LP to up-regulate the expression of the 7 pro-apoptotic proteins Bax (1.3–2.6 folds), cytochrome c (1.4–2.5 folds), DR5 (1.5–2.5 folds), CHOP (1.1–2.3 folds), cleaved caspases-3 (1.5–2.1 folds), cleaved caspases-8 (1.1–1.8 folds), cleaved caspases-9 (1.2–2.8 folds), or to down-regulate the expression of the anti-apoptotic protein Bcl-2 (0.6–0.9 folds). Caspase-3 is an important apoptotic protein in the cells, caspase-3 activation will lead to cell apoptosis. It was observed in these assays that SeLP2 showed a similar capacity as 5-FU to activate caspase-3 and down-regulate procaspase-3. Meanwhile, the classic caspase-3 inhibitor Ac-DEVD-CHO, well-known for its capacity to inhibit the cell apoptosis caused by caspase-3, was also found to antagonize the SeLP2-caused down-regulation of procaspase-3. SeLP2 was verified to have an opposite function than Ac-DEVD-CHO, suggesting the apoptosis induction of SeLP2. Considering that procaspase-3 down-regulation and cleaved caspases-3 up-regulation are essential to activate the apoptotic signaling pathway, it was inferred that SeLP1 and SeLP2 could induce cell apoptosis via activating simultaneously the death receptor (by up-regulating the related proteins DR5 and cleaved caspase-8), mitochondrial-dependent (by up-regulating the related proteins cytochrome c and cleaved caspase-9), and ER stress pathways (by up-regulating the related protein CHOP) (Fig. 8).

4. Discussion

Accumulating evidence indicated that increased intake of natural bioactive substances (e.g. polyphenols and polysaccharides) from various food resources could promote health or prevent cancers.

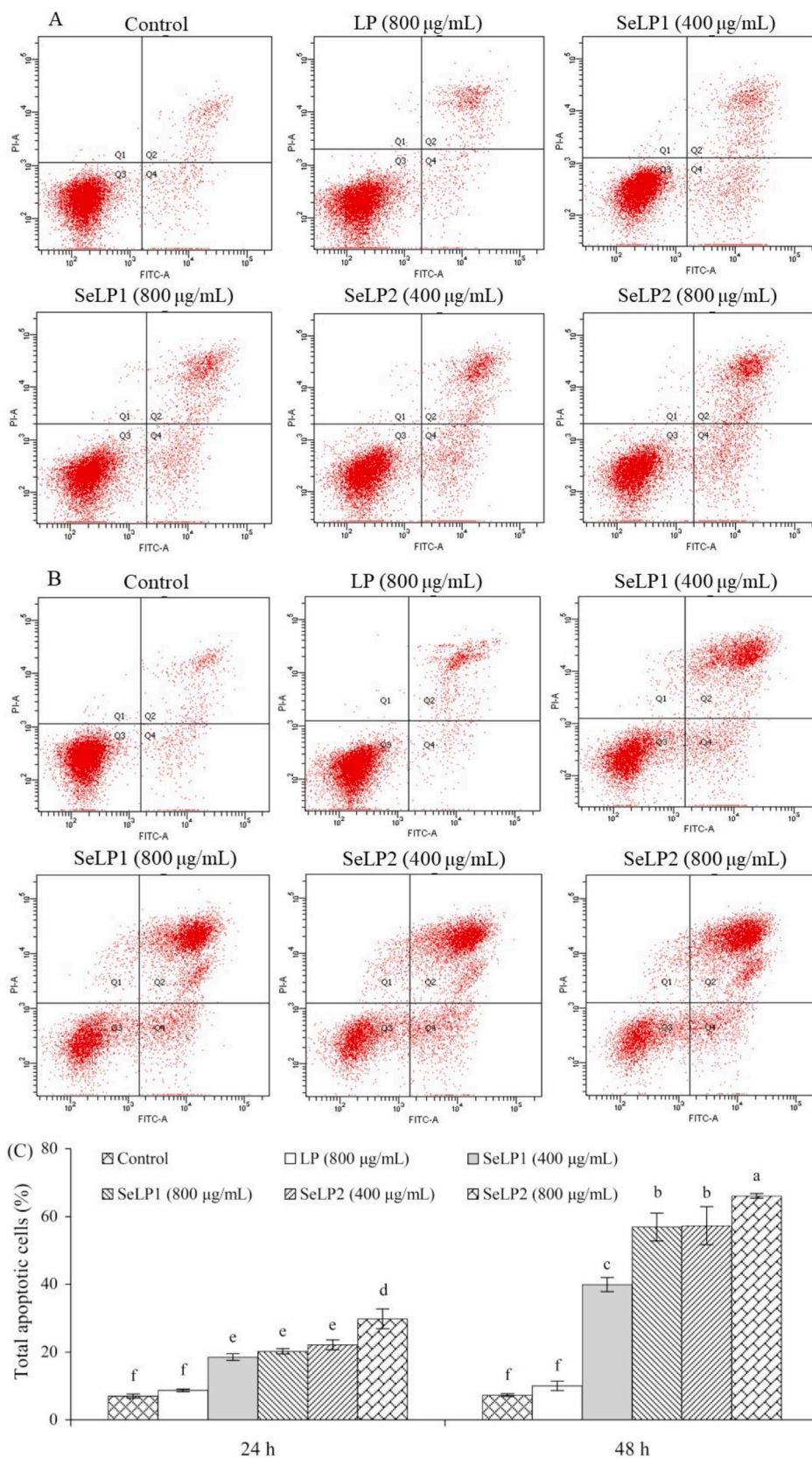


Fig. 6. Apoptosis induction of longan polysaccharides (LP) and selenylated polysaccharide products (SeLP1 and SeLP2) towards the HCT-116 cells with the treatment times of 24 h (A) and 48 h (B), as well the calculated total apoptosis cell percentages (C). Different lowercase letters above the columns indicate significant differences of the mean values ($p < 0.05$).

Table 3

Expression changes of the 8 apoptosis-related genes in HCT-116 cells exposed to longan polysaccharides (LP) and two selenylated polysaccharides (SeLP1 and SeLP2).

Gene	Relative expression fold					
	Control	LP (800 µg/mL)	SeLP1 (400 µg/mL)	SeLP1 (800 µg/mL)	SeLP2 (400 µg/mL)	SeLP2 (800 µg/mL)
Bax	1.0 ± 0.1	1.1 ± 0.0	1.4 ± 0.1	2.1 ± 0.1	2.2 ± 0.0	2.6 ± 0.2
Bcl-2	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.7 ± 0.0
Caspase-3	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
Caspase-8	1.0 ± 0.3	1.1 ± 0.1	1.3 ± 0.1	1.4 ± 0.0	1.4 ± 0.1	1.5 ± 0.1
Caspase-9	1.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.7 ± 0.3	1.7 ± 0.2	1.9 ± 0.2
CHOP	1.0 ± 0.2	1.0 ± 0.0	1.2 ± 0.1	1.4 ± 0.2	1.4 ± 0.1	1.6 ± 0.1
Cytochrome c	1.0 ± 0.1	1.0 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.0	1.5 ± 0.0
DR5	1.0 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	2.0 ± 0.3

Polyphenols and polysaccharides were thus widely studied for their healthcare potential (Tuohy et al., 2012; Khan et al., 2019; Wang et al., 2020b). Dietary polyphenols are generally considered as the promising chemopreventive agents for CRC because they can modulate cell proliferation, apoptosis, and metastasis by targeting a variety of molecules and biochemical pathways related to tumor development (Khan et al., 2021). For example, it was found that resveratrol, quercetin, and xanthohumol were capable of inhibiting the growth of HCT-116, SW480, and HT-29 cells (Araújo et al., 2011), while the exposure of HCT-116 cells to the polyphenols bound with foxtail millet bran led to growth inhibition and cell apoptosis (Shi et al., 2015). Furthermore, the polysaccharides from *Nostoc commune* Vaucher were able to inhibit the growth of HCT-116 and HT-29 cells via activating the NF-κB and AKT/JNK1/2 signaling pathways (Guo et al., 2019), while *Cordyceps sinensis* polysaccharides had growth inhibition on HCT-116 cells via activating the PI3K-AKT-mTOR and AMPK-mTOR-ULK1 signaling pathways (Qi et al., 2020). More importantly, chemical selenylation of peptidoglycans was proven to enhance their anti-tumor effect, because the selenylated peptidoglycans showed greater suppression on HT-29 cells (He et al., 2017). It was also identified that the selenylated *Artemisia sphaerocephala* polysaccharides (Se contents of 4344–13,030 mg/kg) had higher anti-tumor potentials that were positively correlated with Se contents (Liu et al., 2021). It was thus reasonable for the present study that the used chemical selenylation as well as higher selenylation extent made a contribution to the enhanced activities of SeLP1 and SeLP2.

In general, apoptosis is considered as one of the important mechanisms for the inhibition of cancer growth, and can be mediated by these pathways namely death receptor, mitochondrial, and ER stress pathways (O'Brien and Kirby, 2008). It was confirmed that the polyphenols bound with foxtail millet bran induced cell apoptosis by activating the mitochondria-mediated signaling pathway (Shi et al., 2015), while apigenin could induce cell apoptosis via activating the signaling pathways involved in the mitochondria, death receptor, and ER stress (Wang and Zhao, 2017). In addition, selenylated polysaccharides have been verified to cause cell apoptosis in several cancer cells. For example, the Se-enriched *Ganoderma lucidum* and *Ginkgo biloba* polysaccharides could induce apoptosis of the respective MCF-7 and T24 cells via causing MMP disruption and activating the mitochondrial pathway (Shang et al., 2011; Chen et al., 2017), while the selenylated *Artemisia sphaerocephala* polysaccharides could induce apoptosis of HepG2 cells via activating the death receptor signaling pathway (Liu et al., 2021). In general, organic

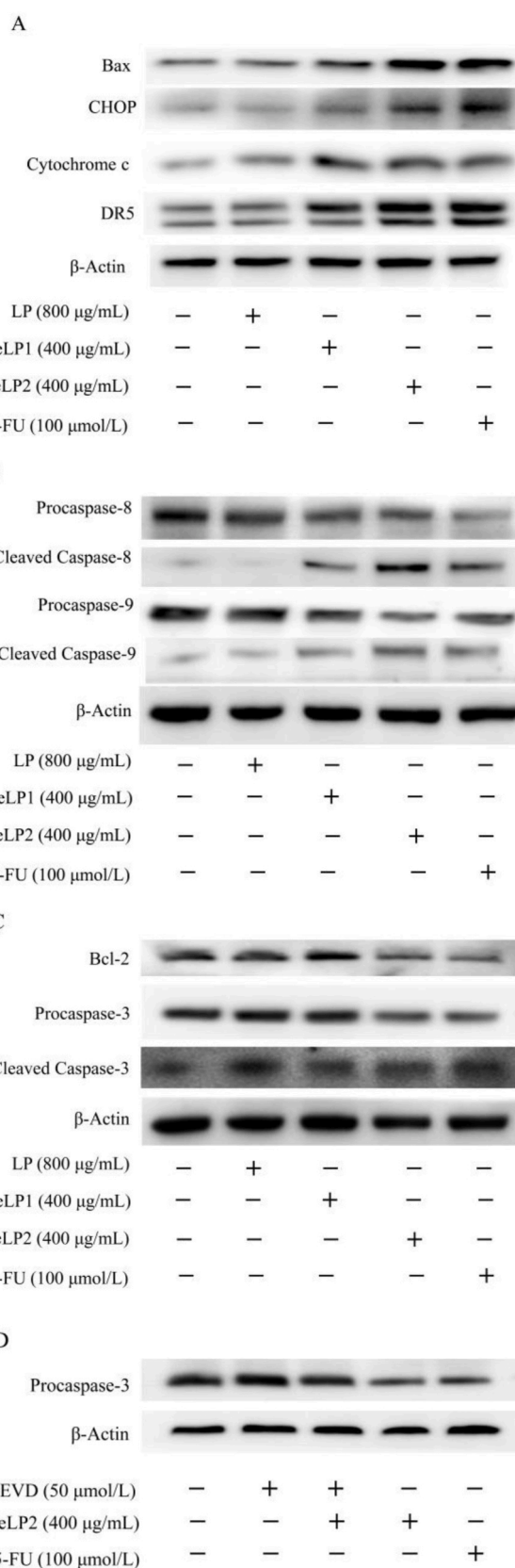


Fig. 7. Western-blotting assays of the apoptosis-related proteins in HCT-116 cells treated with or without longan polysaccharides (LP), selenylated polysaccharide products (SeLP1 and SeLP2) (A), and caspase-3 inhibitor Ac-DEVD-CHO for 48 h (B). 5-FU of 100 µmol/L was used as positive control.

Table 4

Expression changes of the nine apoptosis-related proteins in HCT-116 cells exposed to longan polysaccharides (LP), two selenylated polysaccharides (SeLP1 and SeLP2), inhibitor DEVA, or DEVA plus SeLP2.

Protein	Relative expression fold						
	Control	LP	SeLP1	SeLP2	5-FU	DEVD	DEVD & SeLP2
Bax	1.0 ± 0.1	1.3 ± 0.1	1.7 ± 0.1	2.6 ± 0.3	2.8 ± 0.4	NA	NA
Bcl-2	1.0 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	NA	NA
CHOP	1.0 ± 0.0	1.1 ± 0.2	2.1 ± 0.1	2.3 ± 0.3	2.0 ± 0.4	NA	NA
Cleaved caspase-3	1.0 ± 0.2	1.5 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	3.5 ± 0.7	NA	NA
Cleaved caspase-8	1.0 ± 0.1	1.1 ± 0.2	1.5 ± 0.2	1.8 ± 0.1	1.6 ± 0.1	NA	NA
Cleaved caspase-9	1.0 ± 0.2	1.2 ± 0.1	1.8 ± 0.2	2.8 ± 0.3	2.3 ± 0.3	NA	NA
Cytochrome c	1.0 ± 0.1	1.4 ± 0.1	2.6 ± 0.4	2.5 ± 0.4	2.4 ± 0.4	NA	NA
DR5	1.0 ± 0.3	1.5 ± 0.2	2.4 ± 0.1	2.5 ± 0.4	2.3 ± 0.3	NA	NA
Procaspase-3	1.0 ± 0.1	NA	NA	0.6 ± 0.0	0.5 ± 0.0	1.0 ± 0.0	0.8 ± 0.0

NA, not assessed.

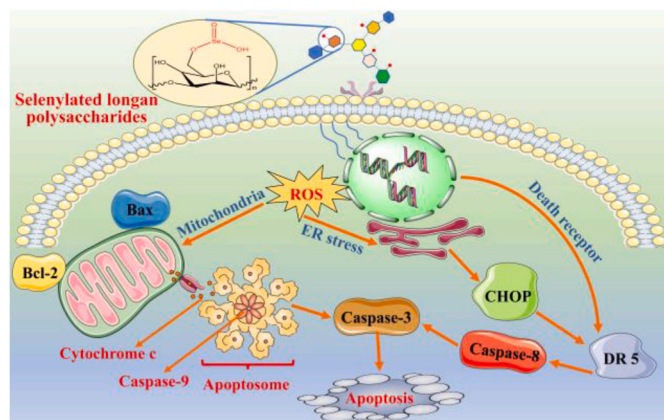


Fig. 8. The underlying ways for the selenylated polysaccharides to induce cell apoptosis in HCT-116 cells.

Se compounds including selenylated polysaccharides can induce the apoptosis of cancer cells effectively (Sinha and El-Bayoumy, 2004; Fernandes and Gandin, 2015; Liu et al., 2000), while the mitochondrial and death receptor pathways are usually involved in the anti-cancer effect of the selenylated polysaccharides (Liu et al., 2021). To the best of our knowledge, selenylated polysaccharides are still not sufficiently clarified for their capacity to cause the ER stress-mediated cell apoptosis, in spite of methylseleninic acid had been revealed to induce the apoptosis of human prostate cancer cells (PC-3) via this pathway (Wu et al., 2005). In consistence with the results of these mentioned studies (Wu et al., 2005; Shi et al., 2015; Wang and Zhao, 2017), the present results indicated that these assessed samples in the cells indeed had growth suppression and apoptosis induction via the mitochondrial and

death receptor pathways. Meanwhile, the present study also revealed that these assessed samples in the cells could induce apoptosis via the ER stress pathway, reflected by the two facts: (1) enhanced ROS formation, Ca^{2+} levels, and MMP loss; (2) up-regulated pro-apoptotic proteins CHOP, DR5, and cleaved caspases-3/-8.

For polysaccharides, their anti-cancer function is associated with their unique chemical characteristics including molecular weights, molecular structures, and monosaccharide types (Xie et al., 2020). Compared with the *Ganoderma lucidum* polysaccharide, the polysaccharide from the whole grass of *Scutellaria barbata* had similar arabinose, galactose, and glucose compositions but possessed higher activity against HT-29 or HCT-116 cells because of their lower molecular weights (Liang et al., 2014; Sun et al., 2017). It was found that the main and side chains of brown alga polysaccharides were linked by respective β -(1 → 3) and β -(1 → 6) glycosidic bonds, and the polysaccharide had an anti-tumor effect on human colon cancer DLD-1 cells (Ermakova et al., 2013). More importantly, the β -(1 → 3) linkages in the main chain and β -(1 → 6) branch chain are important to the anti-cancer activities of polysaccharides (Wasser, 2002). It was detected that LP contained arabinose, mannose, glucose, and galactose, together with the main linkages of (1 → 4)- β -glucose and (1 → 6)- β -mannose (Huang et al., 2019). Thus, LP, SeLP1, and SeLP2 were found to possess these assessed anti-cancer activities to the cells. It is well-regarded that a chemical modification (e.g. phosphorylation, sulfation or selenylation) could alter polysaccharide characteristics and then affect polysaccharide activities (Xie et al., 2020). For example, the sulfated arabinogalactans received altered monosaccharide compositions and increased activities to several cancer cells including A549, MCF-7, and HepG2 cells (Tang et al., 2019), while the sulfated hyperbranched mushroom polysaccharides also obtained higher anti-cancer activity to HepG2 cells (Tao et al., 2006). The selenylated polysaccharides were also proven in four past studies to have better anti-cancer effects on several cancer cells than the parent polysaccharides (Shang et al., 2011; Wang et al., 2016; Wang et al., 2017; Zhou et al., 2020). In the present study, both SeLP1, and SeLP2 after the chemical selenylation had altered saccharide compositions and Se incorporation. Reasonably, SeLP1 and SeLP2 possessed higher activity than the unmodified LP in the cells. In addition, SeLP2 had a higher selenylation extent than SeLP1, and thus showed the highest activities to the cells. Collectively, it is encouraged to develop the Se-rich agricultural products through various technical approaches, to obtain natural polysaccharides with higher Se conjugation, based on the fact that polysaccharides with higher Se content might possess higher healthcare potentials.

5. Conclusion

Our finding highlights that a chemical selenylation of LP caused compositional changes for the resultant selenylated products including much increased Se content, enhanced neutral saccharides but reduced uronic acid and sulfate contents. In addition, the selenylated products possessed higher activity in HCT-116 cells, via causing greater growth inhibition, more morphology alteration, more generation of intracellular ROS and Ca^{2+} , and greater MMP loss. Meanwhile, the selenylated products were more potent to induce cell apoptosis, via up- and down-regulating of the 8 apoptosis-related genes and proteins. Totally, higher selenylation of the selenylated products consistently led to higher activities in the cells. Thus, this chemical selenylation is applicable to endow natural polysaccharides with higher Se content, changed saccharide features, and enhanced activity. Whether this selenylation has a possible impact on other chemical features and activities of LP or other polysaccharides needs future investigation. Furthermore, the specific structural features and possible structure-activity relationship of the selenylated products also need to be elucidated.

CRedit authorship contribution statement

Ya-Hui Yu: Formal analysis, Methodology, Writing – original draft.
Li Wang: Methodology. **Qiang Zhang:** Writing – original draft. **Xiao-Nan Zhang:** Methodology. **Xin-Huai Zhao:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

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.

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