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Polysaccharides from *Annona muricata* leaves protect against cisplatin-induced cytotoxicity in macrophages by alleviating mitochondrial dysfunction

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Abstract. Cisplatin is a prominent chemotherapeutic agent that can induce significant damage to normal cells. Therefore, it is important to develop agents that protect normal cells without influencing the chemotherapeutic effect of cisplatin. The present study was conducted to explore the protective effects of *Annona muricata* leaf polysaccharides (ALPS) against cisplatin-induced toxicity in macrophages. Apoptosis was assessed in macrophages and lung cancer cells to investigate the cytoprotective effect of ALPS, their effect on the production of cisplatin-induced reactive oxygen species (ROS) and the loss of the mitochondrial transmembrane potential (MTP). Cisplatin, when used alone or in combination with ALPS, showed significant toxicity against A549 and H460 lung cancer cells. However, cisplatin-induced cytotoxicity was suppressed by cotreatment of RAW 264.7 macrophages with ALPS. ALPS significantly inhibited the upregulation of BAX, cytosolic cytochrome c and caspases-3, -8 and -9. Moreover, ALPS resulted in the cleavage of PARP and downregulation of Bcl-2 levels in a concentration-dependent manner, which ultimately led to a reduction in the apoptotic and necrotic populations of cisplatin-treated RAW 264.7 macrophages. The suppression of the apoptotic signaling pathways was mediated through the reduction of ROS and MTP loss in cisplatin-treated RAW 264.7 macrophages. In addition, ALPS alleviated cell damage by suppressing the mitochondrial apoptotic pathways in cisplatin-treated bone marrow-derived macrophages. Together, these findings suggested that ALPS may alleviate the toxic side effects of chemotherapeutic agents and act as a potential candidate for use as an effective adjuvant therapy.

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Introduction

Cancer is one of the leading causes of deaths worldwide, and chemotherapy is the main treatment approach for a majority of human cancers (1,2). Epidemiological studies suggest that patients with cancer achieve a substantially longer survival time after treatment with chemotherapeutic agents, such as 5-fluorouracil, paclitaxel, doxorubicin, and cisplatin (CP) (3-5). There have been reports on the potential side effects of chemotherapeutic agents that adversely affect the quality of life of patients with cancer, including oral mucositis, ototoxicity, immunotoxicity, neurotoxicity, hepatotoxicity, and cardiotoxicity (6-10). Thus, it is necessary to investigate potential strategies for effective adjuvant therapy to reduce and prevent the incidence of side effects from chemotherapeutic agents.

Natural products have shown promising potential as supplements for the prevention of chemotherapy-induced side effects (11,12). Polysaccharides derived from vegetables, fruits, and plants, have attractive properties, including low toxicity, high efficacy, and a wide range of sources. Accordingly, the use of active polysaccharides has been discussed in the fields of medicine, functional food, and molecular biology (13). For example, polysaccharides from *Ganoderma atrum* have shown preventative effects against cyclophosphamide-induced myelosuppression and oxidative stress (14). Polysaccharides from *Astragalus* alleviate the paclitaxel-induced cytotoxicity by reversing the changes in cell cycle and apoptosis (15). Recently, we had reported that polysaccharides from the *Cudrania tricuspidata* fruit play an important role in the alleviation of CP-induced cytotoxicity in macrophages and a mouse model (16). These findings suggest that further research

is needed on polysaccharides to assess their role in preventing chemotherapy-induced toxic side effects.

Annona muricata (also known as graviola) has extensively been used as a source of traditional medicine, with a long history of treating various diseases, such as cancer, inflammation, and hypertension (17-19). Our studies have also shown that galactose (68.4%) is the major monosaccharide among the *A. muricata* leaf polysaccharides (ALPS), followed by glucose (24.37%), mannose (9.81%), and other sugars. ALPS exert protective effects against oxidative stress-induced cellular damage and radiation-induced skin injury (20,21). However, there is no evidence supporting the cytoprotective role of ALPS against chemotherapy-induced toxicity.

Therefore, this study aimed to evaluate the cytoprotective effects of ALPS in CP-treated macrophages and to explore the potential use of ALPS as a supplement to reduce immunotoxicity during chemotherapy.

Materials and methods

Preparation of ALPS. Leaves of *A. muricata* were purchased from Todam (Cheonan, Korea). ALPS were prepared following a previously described method (22). Briefly, 50 g of *A. muricata* leaf powder were extracted with 500 ml of deionized water at 100˚C for 2 h. The extract was filtered through Whatman no. 4 filter paper then incubated with five volumes of 70% ethanol overnight at 4˚C. The ethanol phase was centrifuged at 1,700 x g for 20 min to collect the precipitated polysaccharides, which were lyophilized (Hanil, Gwangju, Korea) and dissolved in sterile deionized water.

Reagents and antibodies (Abs). CP and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The fluorescein isothiocyanate (FITC)-conjugated annexin V/propidium iodide (PI) kit was purchased from BD Biosciences (San Diego, CA, USA). Monoclonal primary Abs against cleaved caspase-3 (#9661), cleaved caspase-8 (#9496), cleaved caspase-9 (#9501), cleaved poly (ADP-ribose) polymerase (PARP, #9541), B-cell lymphoma 2 (Bcl-2, #2870), Bcl-2-associated X protein (BAX, #2772), cytochrome c (#12959), and β-actin (#4970), and horseradish peroxidase-conjugated goat anti-rabbit (#7074) and anti-mouse secondary Abs (#91196) were obtained from Cell Signaling Technology (Danvers, MA, USA). 2',7'-Dichlorodihydrofluorescein diacetate (H_2DCFDA) and 3,3'-dihexyloxacarbocyanine ($DiOC_6$) were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Cell culture. Human lung cancer cell lines (A549 and H460) and a murine macrophage cell line (RAW 264.7) were obtained from the Korea Cell Line Bank (Seoul, Korea). Cells were cultured in complete Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum and 100 U/ml penicillin/streptomycin (Gibco BRL), and maintained in a humidified chamber at 37˚C with 5% CO_2.

Measurement of cell viability. A549, H460, and RAW 264.7 cells were seeded in complete DMEM into 96-well plates and allowed to grow to approximately 70-80% confluency at 37˚C with 5% CO_2. The cells were treated with 0-1,000 μg/ml ALPS for 2 h and then incubated with CP (0, 10, 15, and 20 μM) for 24 h. Subsequently, the medium was replaced with MTT solution (0.5 mg/ml in complete DMEM) and incubation continued for 2 h. The solution was aspirated, and the formed formazan crystals were dissolved in 150 μl of dimethyl sulfoxide (Sigma-Aldrich) per well. Absorbance was measured at 570 nm using an Epoch microplate reader (BioTek Instruments, Winooski, VT, USA).

Flow cytometric analysis of apoptosis. The extent of apoptosis was determined by flow cytometry using a FITC-conjugated annexin V/PI kit and a terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. RAW 264.7 cells were incubated in a 48-well plate for 12 h and then treated with CP in the presence or absence of ALPS for 24 h. The cells were harvested and washed with phosphate-buffered saline (PBS; Gibco BRL), resuspended in the binding buffer, and stained with annexin V-FITC and PI for 15 min. The stained cells were analyzed using a FACSVerse™ flow cytometer (BD Biosciences). The TUNEL assay was performed using the DeadEnd™ fluorometric TUNEL system (Promega, Madison, WI, USA) following the manufacturer's instructions. Briefly, the cells were fixed with a 4% formaldehyde solution in PBS for 25 min, then permeabilized with 0.2% Triton X-100 in PBS for 5 min. After washing with PBS, the samples were incubated in the reaction buffer from the staining kit for 60 min. TUNEL-positive cells were analyzed using a FACSVerse™ flow cytometer and FlowJo software (version 10, BD Biosciences).

TUNEL assay using confocal microscopy. RAW 264.7 cells were cultured on glass slides for 12 h, then treated with CP in the presence or absence of ALPS for 24 h. The cells were fixed in 4% paraformaldehyde in PBS for 30 min, then permeabilized in 0.2% Triton X-100/PBS (Sigma-Aldrich, Darmstadt, Germany) for 5 min. After washing the slides twice using PBS and adding 100 μl of the equilibration buffer for 10 min at 4˚C, the samples were incubated in 50 μl of the TdT reaction mixture for 1 h at 37˚C in a humidified chamber in the dark. To stop the reaction, the glass ± diamidino-2-phenylindole was added in the mounting medium and TUNEL-positive cells were analyzed using a LSM510 confocal laser scanning microscope (Carl Zeiss, Jena, Germany).

Isolation of bone marrow-derived macrophages (BMDMs). Five, seven-week-old (18±2 g) female C57BL/6 mice were purchased from Orient Bio (Seoul, Korea). They were acclimated to the temperature (25±2˚C) and humidity (55±5%) of the housing unit and fed a sterile commercial mouse diet and water *ad libitum*. BMDMs were isolated from these mice following an established protocol (23). Specifically, after sacrifice by cervical dislocation, bone marrow cells were isolated from the femur and tibia. Erythrocytes were lysed using a red blood cell lysing buffer (Sigma-Aldrich). Thereafter, BMDMs were plated in a petri dish and differentiated for six days in complete DMEM containing 10 ng/ml macrophage colony-stimulating factor (R&D Systems, Minneapolis, MN, USA). On day 3 of

differentiation, 10 ml of the macrophage complete medium were added. After 6 days of differentiation, the cells were harvested and the $F4/80^+$ and $CD11b^+$ BMDM populations (>90% purity) were isolated with a FACSverse using anti-F4/80 and anti-CD11b Abs (BD Bioscience). The animal experiment was approved by the Institutional Animal Care and Use Committee of the Korea Atomic Energy Research Institute (KAERI-IACUC-2020-005).

Analysis of BMDM apoptosis. On day 7 of differentiation, adherent BMDMs were harvested using a 0.25% trypsin-EDTA solution (Gibco BRL) and plated into 48-well plates in complete DMEM at a density of 10,000 cells/well. The cells were treated with CP in the presence or absence of ALPS for 24 h, then stained with annexin V/PI as described above.

Western blotting. RAW 264.7 cells or BMDMs were seeded into 6-well plates and treated with CP in the presence or absence of ALPS. The cells were lysed in RIPA buffer (Pierce) containing a protease inhibitor cocktail and 1 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich). After centrifugation at 16,000 x g for 20 min at 4˚C, the total protein concentration was determined in the supernatant using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific). The cell lysates (20 μg of protein) were resolved by 10-12% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the separated proteins were electrotransferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat milk and incubated with primary Abs (diluted 1:1,000) against cleaved caspases-3, -8, and -9, cleaved PARP, BAX, Bcl-2, cytochrome c, and β-actin overnight at 4˚C. After washing, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary Abs (diluted 1:5,000) for 1 h. Protein bands were visualized using the Pierce ECL western blotting substrate (Thermo Fisher Scientific).

Measurement of reactive oxygen species (ROS). Intracellular ROS levels were measured using the H2DCFDA assay (24). After incubation with CP and ALPS, 10 μM H2DCFDA was added to the cells for 30 min at 37˚C in the dark, and the cells were detached from the plates. After the cells were washed twice with PBS, the fluorescence intensity of the oxidized DCF was detected using a FACSVerse™ flow cytometer and FlowJo software.

Measurement of the mitochondrial transmembrane potential (MTP). Loss of the MTP was analyzed using $DiOC_6$. Cells were incubated with 10 nM $DiOC_6$ in fresh medium for 30 min at 37˚C in the dark, washed, and resuspended in PBS. The fluorescence intensity of DiOC6 was detected using a FACSVerse™ flow cytometer and FlowJo software.

Statistical analyses. All experiments were repeated three times using triplicate wells. Statistical significance was analyzed by a one and two-way analysis of variance followed by Tukey's test using Prism version 8.0 software (GraphPad Software, San Diego, CA, USA). The results are expressed as means ± standard deviation. Values of $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ were considered statistically significant.

Results

Effects of ALPS on CP-induced toxicity in lung cancer cells and RAW 264.7 macrophages. To identify the mitigating effect of ALPS in CP-induced cytotoxicity, RAW 264.7 macrophages- and human lung cancer cell lines (A549 and H460) were used to demonstrate the effect of ALPS on normal and cancer cells. To determine the appropriate concentration of ALPS, ALPS cytotoxicity was first evaluated against RAW 264.7 macrophages. As shown in Fig. 1A, ALPS did not exert cytotoxicity at the concentration range of 15.6-500 μg/ml in RAW 264.7 macrophages. Next, we investigated whether ALPS affected the viability of CP-treated lung cancer (A549 and H460) cells and RAW 264.7 macrophages. In A549 and H460 cells, both CP alone (10, 15, and 20 μM) and in combination with ALPS (15.6, 31.25, and 62.5 μg/ml) resulted in a concentration-dependent inhibition of tumor cell growth compared with that in the control (CP-untreated) group. These results were in agreement with supplement effect of ALPS on CP-induced ROS production and MTP loss in lung cancer cells (Figs. S1 and S2). Meanwhile, the CP-induced cytotoxicity was effectively suppressed by treatment of RAW 264.7 macrophages with ALPS at various concentrations (15.6, 31.25, and 62.5 μg/ml) compared with that in the CP alone group (Fig. 1B). Furthermore, at concentrations of ALPS higher than 62.5 μg/ml, the cytoprotective effect was similar to the concentration at 62.5 μg/ml of ALPS in cisplatin-treated RAW 264.7 macrophage (data not shown). Based on the results obtained, the appropriate concentrations for ALPS (31.25 and 62.5 μg/ml) and CP (15 μM) were determined and used in all subsequent experiments.

Inhibitory effects of ALPS on CP-induced apoptotic cell death in RAW 264.7 macrophages. To further confirm that treatment with ALPS resulted in a concentration-dependent increase in the viability of CP-treated RAW 264.7 macrophage cells, the cytoprotective effects of ALPS against CP-induced apoptosis were evaluated using annexin V/PI or TUNEL staining. As shown in Fig. 2A, pretreatment with ALPS (31.25 and 62.5 μg/ml) resulted in a significant increase in cell viability compared with that in the CP only group ($P<0.001$). Furthermore, the CP only treated group showed an increased number of TUNEL-positive cells compared with that in the CP-untreated group, whereas the number was significantly ($P<0.001$) reduced by pretreatment with ALPS (31.25 and 62.5 μg/ml) in CP-treated RAW 264.7 macrophages (Fig. 2B). These results were consistent with the confocal microscopic analysis via TUNEL staining (Fig. 2C). Collectively, these results demonstrated that ALPS attenuated the apoptotic cell death of CP-treated RAW 264.7 macrophages.

Inhibitory effects of ALPS on the CP-induced apoptotic signaling pathways in RAW 264.7 macrophages. To elucidate the possible molecular pathways involved in the cytoprotective action of ALPS against CP-induced apoptosis, we examined the expression of the proapoptotic BAX and antiapoptotic Bcl-2 proteins, as well as that of PARP, cytochrome c, caspases-3, -8, and -9, in the CP-treated RAW 264.7 macrophages. As shown in Fig. 3A and B, exposure to CP resulted in increased levels of cleaved caspases-3, -8, and -9 in RAW 264.7 macrophages.

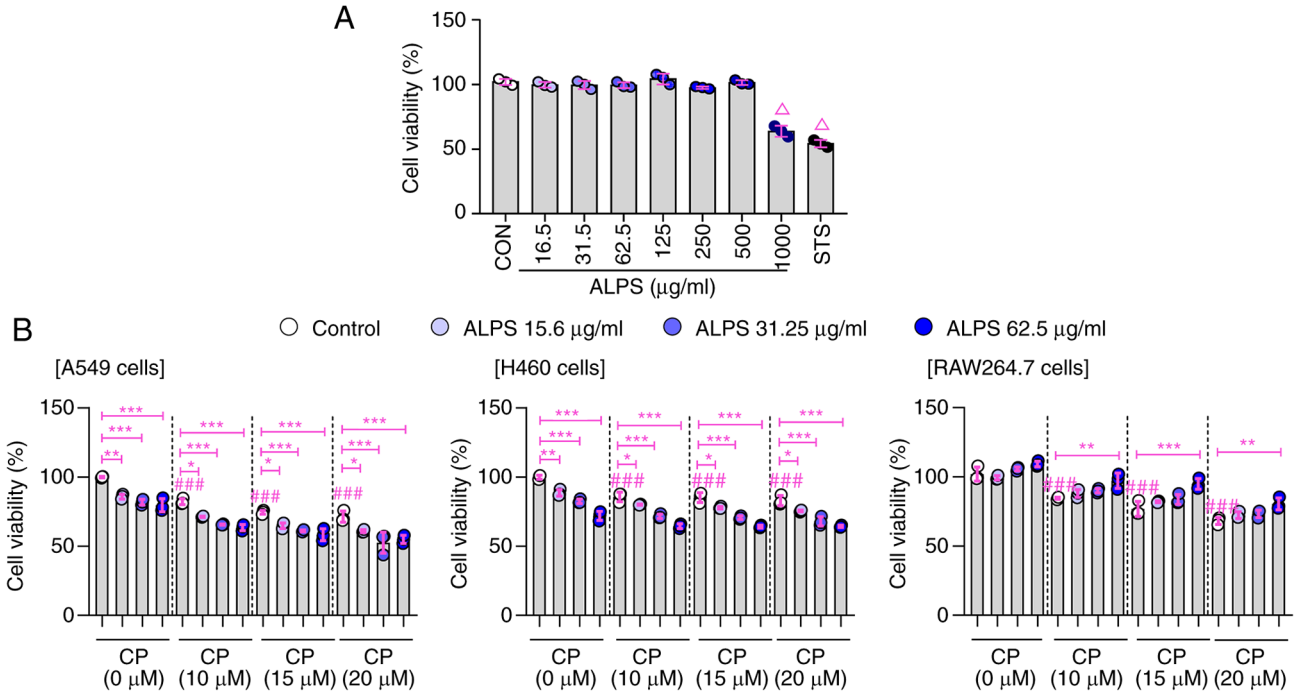

Figure 1. Effects of ALPS on the viability of CP-treated lung cancer cells (A549 and H460) and RAW 264.7 macrophage. (A) Viability of RAW 264.7 cells incubated with various concentrations of ALPS (16.5-1,000 μg/ml) for 24 h. (B) After pre-stimulation of ALPS for 2 h, the cellular viability was assessed in the presence of CP (10, 15 and 20 μM) for 24 h. $^{\Delta}$P<0.001 vs. control group (one-way ANOVA, followed by Tukey's post-hoc test); $^{\#\#\#}$P<0.001 vs. CP (0 μM) treated control group (two-way ANOVA, followed by Tukey's post-hoc test); *P<0.05, **P<0.01 and ***P<0.001 vs. CP-only treated group (one-way ANOVA, followed by Tukey's post-hoc test). ALPS, *Annona muricata* leaf polysaccharides; CP, cisplatin; CON, control.

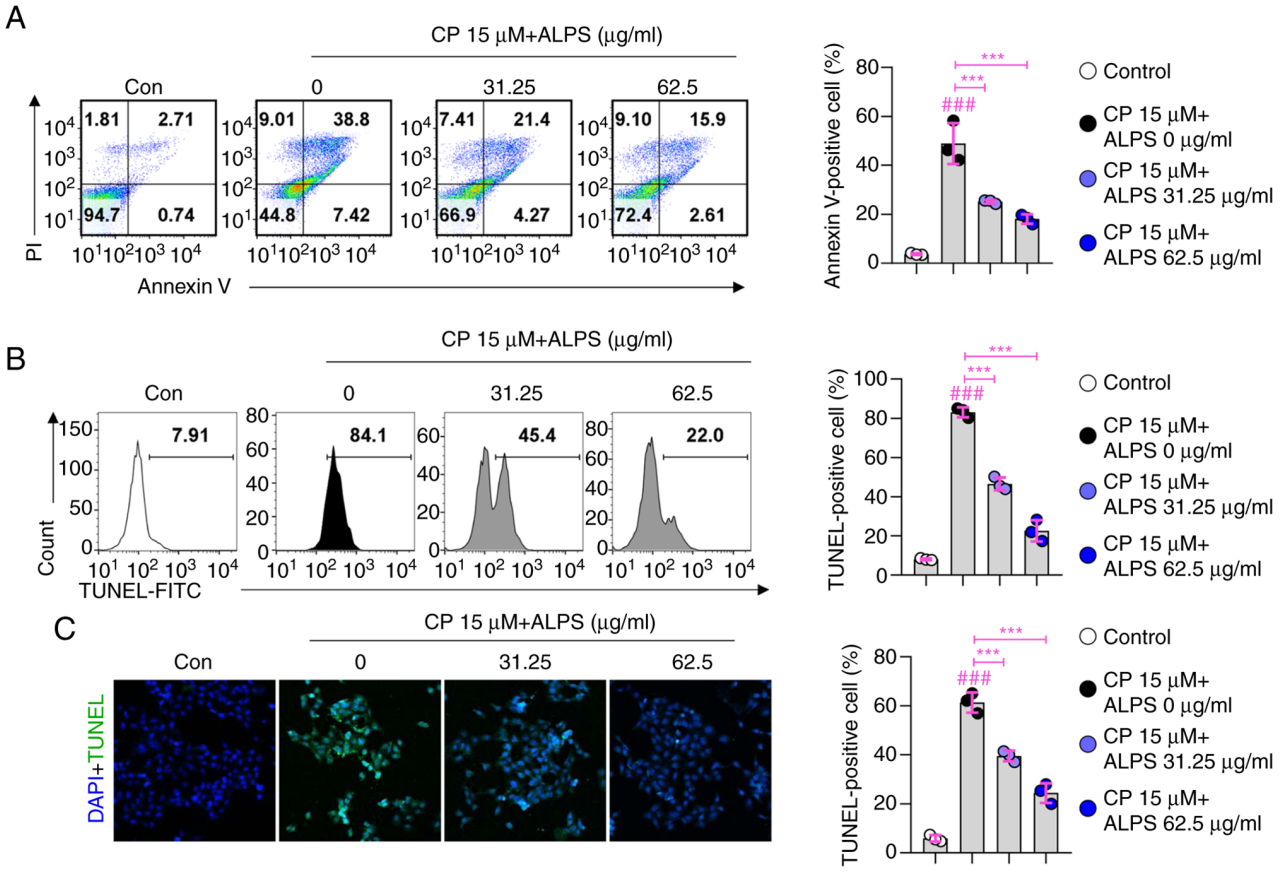

Figure 2. Effects of ALPS on CP-induced apoptosis in RAW 264.7 cells. RAW 264.7 cell pretreated with ALPS (31.25 and 62.5 μg/ml) for 2 h and then exposed to CP (15 μM) for 24 h. Cell death was assessed by flow cytometry after (A) Annexin V/PI staining. TUNEL-positive cells were analyzed by (B) flow cytometry and (C) confocal microscopy. $^{\#\#\#}$P<0.001 vs. control group (one-way ANOVA, followed by Tukey's post-hoc test); ***P<0.001 vs. CP-only treated group (one-way ANOVA, followed by Tukey's post-hoc test). ALPS, *Annona muricata* leaf polysaccharides; CP, cisplatin; Con, control.

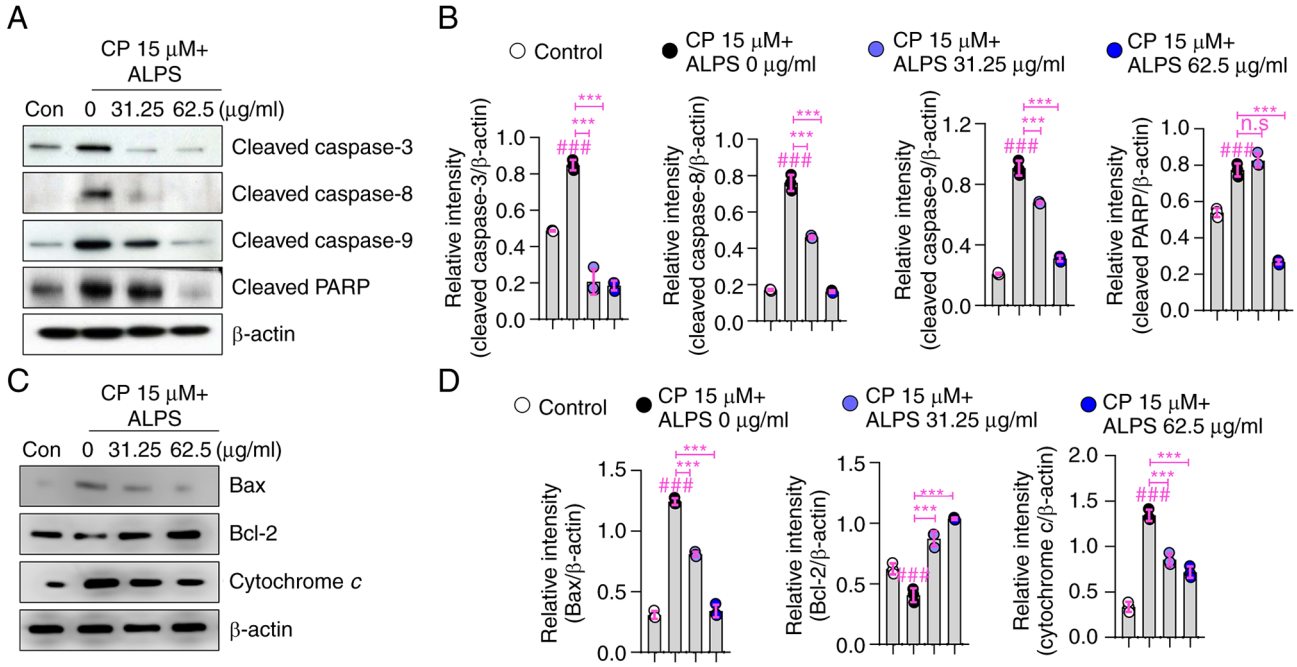

Figure 3. Effects of ALPS on CP-induced apoptotic signaling pathways in RAW 264.7 macrophage. Cells were pretreated with ALPS (31.25 and 62.5 μg/ml) for 2 h and then exposed to CP (15 μM) for 24 h. Expression levels of (A) cleaved caspase-3, cleaved caspase-8, cleaved caspase-9, cleaved PARP followed by (B) densitometric analysis of relative band intensity. (C) Expression levels of Bax, Bcl-2 and cytochrome c followed by (D) densitometric analysis of relative band intensity. $^{###}$P<0.001 vs. control group (one-way ANOVA, followed by Tukey's post-hoc test); ***P<0.001 vs. CP-only treated group (one-way ANOVA, followed by Tukey's post-hoc test). ALPS, *Annona muricata* leaf polysaccharides; CP, cisplatin; Con, control.

In contrast, the levels of cleaved caspases-3, -8, and -9 were noticeably reduced in the CP-treated cells after pretreatment with ALPS (31.25 and 62.5 μg/ml) (P<0.001). Furthermore, PARP cleavage was higher in the CP-treated group than in the control group, whereas pretreatment with ALPS significantly (P<0.001) suppressed PARP cleavage in CP-treated RAW 264.7 macrophages.

Next, we investigated the involvement of BAX, Bcl-2, and cytosolic cytochrome c in the cytotoxic effects observed in CP-treated RAW 264.7 macrophages. The CP only treated group showed upregulation of BAX and cytosolic cytochrome c and downregulation of Bcl-2, whereas pretreatment with ALPS (31.25 and 62.5 μg/ml) significantly (P<0.001) reduced the expression of BAX and cytosolic cytochrome c and increased Bcl-2 expression (Fig. 3C and D). These findings suggested that ALPS inhibited the apoptotic cascade involved in CP-induced cell death, thereby promoting macrophage survival through a cytoprotective action.

Effects of ALPS on ROS production and MTP loss in CP-treated RAW 264.7 macrophages. Next, we examined whether the cytoprotective action of ALPS (31.25 and 62.5 μg/ml) is associated with ROS generation and MTP loss in CP-treated macrophages. As shown in Fig. 4A, treatment of macrophages with CP (15 μM) resulted in an increase in the ROS levels compared with those in the control group, whereas this increase was significantly attenuated by pretreatment of macrophages with ALPS (31.25 and 62.5 μg/ml) (P<0.001). In addition, a significant MTP loss was observed in CP-treated RAW 264.7 macrophages (Fig. 4B), which was attenuated by pretreatment with ALPS (31.25 and 62.5 μg/ml) (P<0.001). These findings suggested that the ALPS-induced cytoprotective effect was due to the inhibition of the apoptotic cascade by reducing CP-induced ROS production and MTP loss.

Protective effects of ALPS against CP-induced apoptotic cell death of BMDMs. The cytoprotective effects of ALPS against CP-induced apoptosis were further elucidated using normal primary BMDMs. Consistent with the results obtained using RAW 264.7 macrophages, pretreatment with ALPS (31.25 and 62.5 μg/ml) induced a significant increase in BMDM viability compared to that of BMDMs treated with CP alone (P<0.001; Fig. 5A). Additionally, pretreatment with ALPS (31.25 and 62.5 μg/ml) significantly inhibited the CP-triggered activation of caspases-3 (P<0.001), -8 (P<0.01), and -9 (P<0.05) in BMDMs, thereby attenuating the apoptotic cell death (Fig. 5B). These findings suggest that ALPS might act as an effective adjuvant therapy against the toxic side effects induced by the chemotherapeutic agents.

Discussion

CP, a DNA targeting agent that forms toxic platinum DNA adducts, is one of the most effective and widely used anticancer agents (25). CP also induces direct damage to the mitochondrial DNA, resulting in oxidative stress by increasing the intracellular ROS level (26). CP-induced oxidative stress contributes to a higher toxicity in tumors and causes damage to the normal tissues owing to its non-selectivity (27). CP induces various side effects, such as myelosuppression, hepatotoxicity, nephrotoxicity, and immunotoxicity (28,29). As these adverse effects can reduce the efficiency of chemotherapy, combination therapy using natural products could be a novel strategy

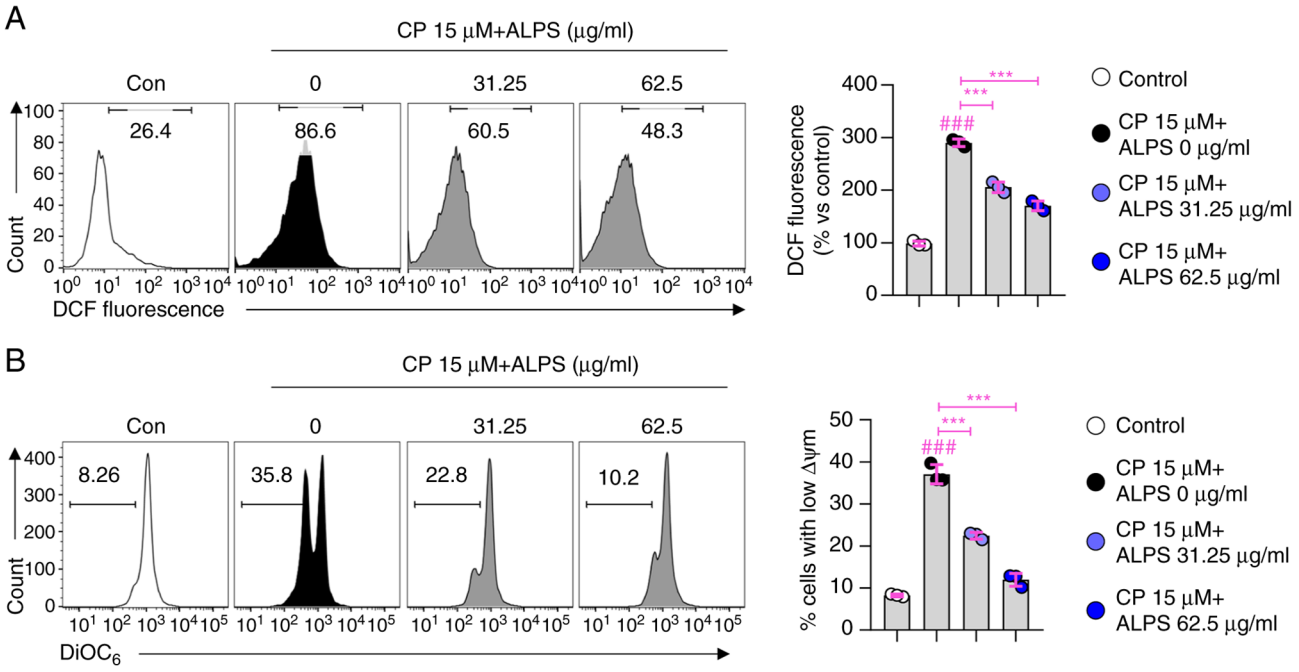

Figure 4. Effects of ALPS on CP-induced ROS production and loss of mitochondrial MTP in RAW 264.7 cells. Cells were pretreated with ALPS (31.25 and 62.5 μg/ml) for 2 h and then exposed to CP (15 μM) for 24 h. (A) H2DCFDA (10 μM) and (B) $DiOC_6$ (10 nM) staining. $^{###}$P<0.001 vs. control group (one-way ANOVA, followed by Tukey's post-hoc test); ***P<0.001 vs. CP-only treated group (one-way ANOVA, followed by Tukey's post-hoc test). ALPS, *Annona muricata* leaf polysaccharides; CP, cisplatin; Con, control; ROS, reactive oxygen species; MTP, transmembrane potential.

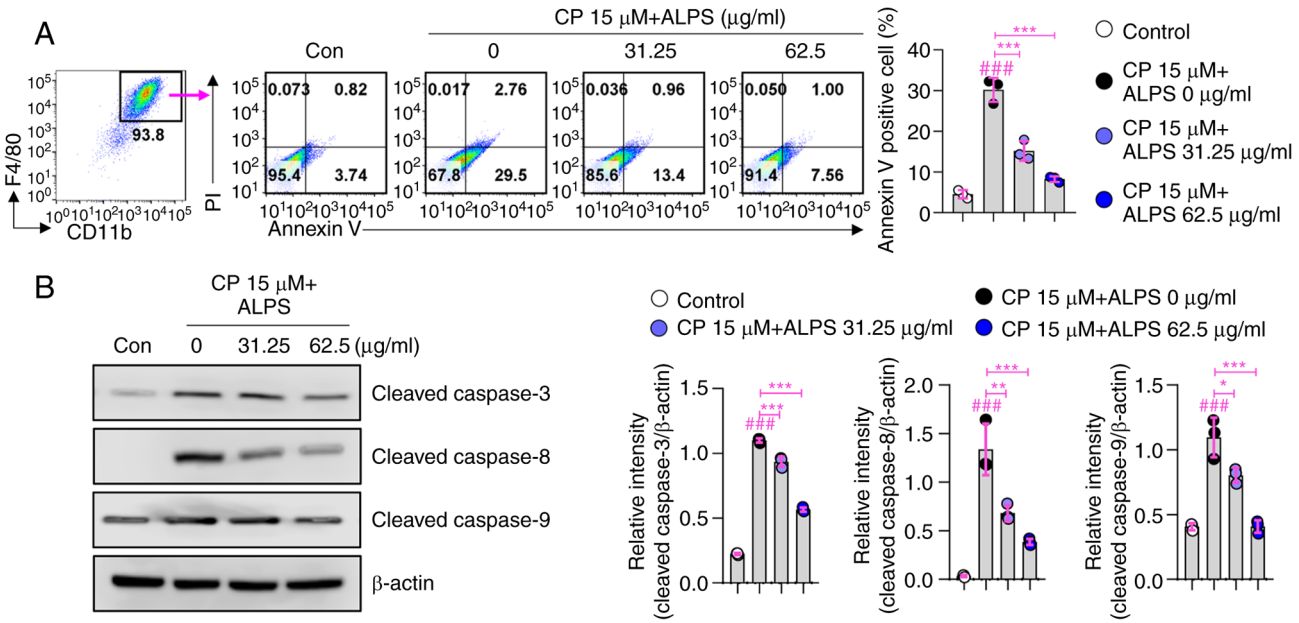

Figure 5. Effects of ALPS on CP-induced apoptosis in BMDMs. BMDMs were pretreated with ALPS (31.25 and 62.5 μg/ml) for 2 h and then exposed to CP (15 μM) for 24 h. (A) Flow cytometric analysis with annexin V/PI staining. (B) Levels of cleaved caspase-3, cleaved caspase-8 and cleaved caspase-9 measured using western blotting and densitometric analysis of relative band intensity. $^{###}$P<0.001 vs. control group (one-way ANOVA, followed by Tukey's post-hoc test); *P<0.05, **P<0.01 and ***P<0.001 vs. CP-only treated group (one-way ANOVA, followed by Tukey's post-hoc test). ALPS, *Annona muricata* leaf polysaccharides; CP, cisplatin; CON, control; BMDMs, bone marrow-derived macrophages.

against CP-induced side effects (30). Herein, we showed that the treatment with CP alone and in combination with ALPS resulted in a concentration-dependent inhibition of the growth of tumor cells, whereas the CP-induced cytotoxicity was effectively alleviated by pretreatment of RAW 264.7 macrophages with ALPS.

CP induces ROS generation to activate the pro-apoptotic proteins and induce the translocation of BAX to the mitochondrial outer membrane, thus releasing cytochrome c into the cytosol (31,32). The apoptosis signal from BAX then initiates the activation of caspase-9 and stimulates the activation of the downstream caspase-3 causing apoptosis

by cleavage of PARP, which acts as a DNA repair agent (33). Therefore, we investigated whether ALPS could alleviate the immune cell toxicity by reducing the CP-induced oxidative stress. First, we observed that ALPS effectively protected the macrophages from the CP-induced apoptosis without loss of toxicity against the lung cancer cell lines. Next, we revealed that ALPS significantly suppressed the apoptotic cascade in the CP-treated RAW 264.7 cells via the upregulation of BAX, cytosolic cytochrome c, and caspases-3, -8, and -9, as well as PARP cleavage and downregulation of Bcl-2.

The induction of the apoptotic signaling pathways is closely associated with the mitochondrial function and ROS production, which are related to various pathological processes such as cellular apoptosis (34-36). Consistently, our results showed that the cytoprotective activity of ALPS was associated with the suppression of the apoptotic signaling pathways via reduction of MTP loss and ROS production in the CP-treated RAW 264.7 cells. In addition, ALPS exerted the cytoprotective effects in the BMDMs via suppressing the caspase signaling pathway. These findings suggest that ALPS may be a potential supplement to alleviate the adverse effects of the chemotherapeutic drugs.

In conclusion, the present study provides strong evidence that ALPS exert cytoprotective effects against CP-induced cytotoxicity in macrophages and could be considered as a potential candidate for combination chemotherapy with CP. In the future, we aim to identify the physicochemical properties of ALPS, as its function can vary depending on the composition and structure of the polysaccharides. We also aim to elucidate the chemoprotective roles of ALPS in the CP-mouse model.

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Availability of data and materials

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

Authors' contributions

JH wrote the paper and analyzed the data. HS, KK and SP acquired the data. WP analyzed the data. EBB designed the study. EHB wrote the paper and designed the study. EBB and EHB confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experimental procedure for the animal study was approved by the Institutional Animal Care and Use Committee of the Korea Atomic Energy Research Institute (approval no. KAERI-IACUC-2020-002).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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