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Regular paper

Ganoderma lucidum polysaccharide inhibits the proliferation of leukemic cells through apoptosis

Mingxing Zhong[1], Jiwei Huang[1], Pingxiang Mao[2], Chengming He[1], Dongmei Yuan[1], Changkun Chen[1], Haiying Zhang[1], Jing Hu[1] and Jingdong Zhang[1]✉

[1]Department of Hematology, Ganzhou People's Hospital, Ganzhou, China; [2]Laboratory Department, Yifeng County Maternal and Child Health Care Hospital, Yifeng, China

Objective: To investigate the cytotoxic effect of polysaccharides derived from *Ganoderma lucidum* on T lymphocyte leukemia cells. Methods: Water-soluble polysaccharides were extracted from the fruit bodies of *G. lucidum*, purified, and characterized using HPGPC-MALLS and NMR. The cytotoxicity of *G. lucidum* polysaccharide fraction 5 (GLP5) to T lymphocyte leukemia cell line Jurkat and human immortalized epidermal cell line HaCat was assessed using MTT assay. Apoptosis was assessed using flow cytometry. Expressions of apoptosis-related genes in the cells after being exposed to GLP5 were detected using Western blot assay. Results: GLP5 was a β-(1→3) and β-(1→6) linked glucan. It inhibited the proliferation of Jurkat cells in a concentration-dependent manner and the half-maximal inhibitory concentration (IC_{50}) was 34.5 mg/L but did not suppress the growth of HaCat cells. Apoptotic cells in Jurkat cells were detected to increase with increasing GLP5 concentrations. The expression levels of cleaved caspase-3 were significantly higher after the cells were exposed to 25 and 50 mg/L GLP5 when compared to non-exposed cells (Control). In addition, the expression levels of BAX and Bcl2 were significantly up- and down-regulated after treatment with GLP5 at 25 and 50 mg/L when compared with control ($P<0.05$), respectively. Conclusions: GLP5 has antiproliferative activity against Jurkat cells and the activity is likely mediated through the activation of apoptosis pathways.

Keywords: *Ganoderma lucidum*, polysaccharide, leukemia, Jurkat, cytotoxicity, apoptosis

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✉e-mail: zhangjingdong_dr@yeah.net
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Abbreviations: AML, acute myeloid leukemia; BAX, BCL-2-associated X apoptosis regulator; COX-2, cyclo-oxygenase-2; CR3, complement receptor; GLP5, *G. lucidum* polysaccharide; IC50, half-maximal inhibitory concentration; LAK, lymphokine-activated killer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 3-NC, 2-amino-4-(3-nitrophenyl)-3-cyano-7-(dimethylamino)-4H-chromene NK: natural killer; OD, optical density; p-Akt1, phosphoserine/threonine kinase 1; P-ERK, phospho-extracellular regulated protein kinases; PS, phosphatidyl-serine; PSP, polysaccharides from *Spirulina platensis;* PVDF, polyvinylidene fluoride; RPMI, Roswell Park Memorial Institute; S.D., standard deviation; SDS, sodium dodecyl sulfate; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; VDAC, voltage-dependent anion channel

INTRODUCTION

Leukemia is a group of malignant hematological diseases. Among them, acute myeloid leukemia (AML) is the most common leukemia in adult population with a prevalence rate between 3 and 5 in 100000 people (Bray *et al*., 2018; Deak *et al*., 2021; Ranta *et al*., 2017). Currently, AML is mainly treated using stem cell transplantation and chemotherapy (Murphy & Yee, 2017; Takami, 2018), which are very expensive and have serious adverse reactions (Bewersdorf *et al*., 2019). Despite substantial advances in our understanding of AML, patient survival remains unsatisfactory, especially within the older age group. The 5-year survival rate after treatments is less than 50% (Hoseini & Cheung, 2017a; Hoseini & Cheung, 2017b; Maia Rda & Wunsch Filho, 2013). Therefore, there is an urgent need to develop efficient, low in toxicity, economic, and targeted drugs for treatment of leukemias (Boudny & Trbusek, 2020)

The mushroom *Ganoderma lucidum* is a basidiomycete rot macrofungus with a potent pharmacological value (Sanodiya *et al*., 2009). Polysaccharides are the main components of *G. lucidum* and have a wide range of pharmacological activities, such as antibacterial, antitumor, and antioxidant activity (Sanodiya *et al*., 2009; Sohretoglu & Huang, 2018). The bioactivity of polysaccharides is closely related to their molecular structures and chemical properties, such as molecular weight, sulfate content, water solubility, conformation, and sugar chain type (Li *et al*., 2013). Low molecular weight polysaccharides from *G. lucidu* (GLP) have been shown to be cytotoxic to various types of tumors, such as colon cancer, ovarian cancer, and prostate cancer (Amini *et al*., 2019; Kladar *et al*., 2016). In addition, polysaccharides from *Spirulina platensis* (PSP) can increase the activity of natural killer (NK) cells in patients with leukemia but did not affect the activity in healthy people. PSP also increases the activity of lymphokine-activated killer (LAK) cells in patients with leukemia, leading to a reduced use of interleukin (IL)-2 (Zeng *et al*., 2000a; Zeng *et al*., 2000b). A fraction of GLP was shown to be able to induce macrophage-like differentiation in human leukemia cells via caspase and p53 activation (Hsu *et al*., 2011), enabling potential leukemia differentiation therapy. In addition, GPL may exert an anti-tumor effect by stimulating the release of cytokines from activated macrophages (Wang *et al*., 1997). However, whether GPL has cytotoxicity against leukemia cells is largely unclear.

In the present study, we aimed to investigate the cytotoxicity of GPL to human leukemia cell line Jurkat and examine the possible mechanisms underlying the activity.

The findings would help develop potential therapeutic agents for leukemia.

MATERIALS AND METHODS

G. lucidum and cell lines

Dry powder of *G. lucidum* fruiting bodies was purchased from Tuohai Biotech, Hebei, China. Human acute T cell leukemia cell line Jurkat, clone E6-1 (Cat. no. TIB152) purchased from American Type Collection Center (ATCC), and human immortalized epidermal cell line HaCat purchased from Weipin Biotech, Shanghai, were cultured in RPMI-1640 medium (Cat. no. 11875119, Gibco, USA) with 10% fetal bovine serum (FBS) (Cat. no. 14190-149, Gibco), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C and 5% CO_2.

Reagents and instruments

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and sodium dodecyl sulfate (SDS) were purchased from Dingguo Changsheng Biotech, Beijing, China; penicillin and streptomycin mixture was purchased from Biyuntian Biotech, Beijing, China; PVDF membrane was purchased from Sigma, St. Luis, USA; antibodies against cleaved caspase-3 (Cat. no. ab32042, 1:1500), Bax (Cat. no. ab32503, 1:1000), Bcl2 (Cat. no. ab32124, 1:2500), and GAPDH (Cat. no. ab253778, 1:1000) were purchased from Abcam, USA; and HRP labeled-goat anti mouse IgG secondary antibody (Cat. no. 32430; 1:2000) and HRP labeled-goat, rabbit IgG secondary antibody (Cat. no. 31460, 1:2000), BCA protein assay (Cat. no. 23227), Dead Cell Apoptosis Kit with Annexin V FITC, and PI (Cat. no. V13242) and ultrasensitive luminescent solution (Cat. no. RF239676) were obtained from Thermo Fisher, USA. Microplate reader (GloMax Discover) was obtained from Promega, USA; vertical laminar flow cabinet (OptiMair) and inverted fluorescence microscope (Revolve FL) were purchased from Esco Lifesciences, Beijing, China; DEAE-52 and Sephadex G-100 were purchased from Whatman (Maidstone, Kent, UK) and Pharmacia (Sweden); 1260 Infinity II LC System was obtained from Agilent, Santa Clara, USA.

Preparation of GLP

GLP was prepared and characterized as previously reported (Zhao *et al*., 2010). Briefly, 100g powder was degreased by soaking in 500 ml 95% ethanol for 24 h on a rotary shaker operated at 80 rpm at room temperature. The degreased power was dried at room temperature for 6 h and extracted with distilled water (300 ml) on a rotary shaker operated at 80 rpm at room temperature for 4 h. The aqueous extract was centrifuged at 500×*g* at room temperature for 20 min to pellet undissolved debris. The supernatant was vacuum-dried at 4°C for 24 h, washed with 200 ml of anhydrous ethanol, acetone, and ether to generate the crude extracts. The crude polysaccharide was dissolved in 50 ml distilled water and applied to a DEAE-52 cellulose column (2.6×30 cm) equilibrated with distilled water and eluted with distilled water and different concentrations of NaCl solution. The elutes were concentrated and further fractionated using size-exclusion chromatography on a Sephadex G-100 column (2.6×60 cm) eluted with 0.01–1 M NaCl at a flow rate of 0.5 ml/min.

Determination of relative molecular mass and molecular radius

High-performance gel permeation chromatography-multi-angle laser light scattering (HPGPC-MALLS) was used to determine the relative molecular mass and molecular radius. The liquid chromatography contained a SB-806HQ Shodex OH pak column (300 mm×8 mm, 6 μm) and a mobile phase of 0.1mol/l Na_2SO_4 flowing at 0.6 ml/min. The column temperature was set at 35°C with 100 μl injection volume filtered through a membrane of 0.22 μm.

NMR spectrum analysis

20 mg GLP was dissolved in 500 μl D20 and frozen-dried three times. It was then dissolved in 500 μl D2O and analyzed at Biotech-pack Inc., Beijing on an Agilent DD 500 MHz superconducting NMR spectrometer to obtain NMR spectrums for ^{1}H and ^{13}C, ^{1}H-^{1}H COSY spectrums and ^{1}H-^{13}C HSQCH spectrums at 25°C. Deuterated DMSO was used as an internal standard.

In vitro cytotoxicity assay

HaCat and Jurkat cells in the logarithmic growth phase after the third passage in RPMI medium containing 10% FBS were used for the experiments. The cells were inoculated into RPMI medium containing different concentrations of GLP (0 to 1000 mg/l) in the wells of 96 well plates and cultured at 37°C and 5% CO_2 for 48 h. 20μl MTT (5 g/l) was added, and the cells were cultured for another 4 h before being used for optical density (OD) assessment at 570 nm according to the manufacturer's instructions.

Flow cytometry apoptosis assays

Flow cytometry analyses were used to assess apoptotic cells after double staining with FITC-Annexin V and PI included in the Dead Cell Apoptosis Kit according to the supplier's instructions. HaCat and Jurkat cells were cultured for 48 h with indicated concentrations of GLP5. The cells (both treated and untreated) were harvested and rinsed twice with PBS, then resuspended in 100 μl binding buffer included in the kit. 10 μl FITC-Annexin V was added to the cells followed by the addition of 10 μl PI. The samples were then incubated for 10 min in the dark at 4°C and loaded onto a flow cytometer (Becton Dickinson FACS) for assessment.

Western blot

500 μL Jurkat cells (2×10^6 cells/mL) in the logarithmic growth phase were cultured in RPMI medium containing 10% FBS and GLP at 37°C and 5% CO_2 for 48 h. The cells were pelleted by centrifugation at 500×*g* at room temperature for 10 min and lysed in RIPA buffer. The extracted proteins were quantified by the Bradford method using a BCA kit according to the supplier's instructions. 40 μg proteins were subjected to 12% SDS-PAGE and transferred to PVDF membranes. After the membranes were blocked by 5% defatted milk for 1 h at room temperature, they were incubated with anti-cleaved caspase, anti-BAX, and anti-Bcl2 antibodies at 4°C overnight and secondary antibodies (goat anti-rabbit or anti-mouse IgG secondary antibodies) at room temperature for 4 h. Enhanced chemiluminescence solution was then drop-added to the membranes and immunoreactive bands were captured with the gel imaging system and analyzed for gray value with the "Image Lab" software.

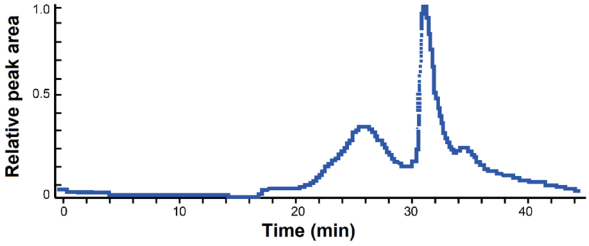

Figure 1. HPGPC-MALLS analysis of GLP5 on a SB-806HQ Shodex OH pak with a mobile phase of 0.1mol/L Na2SO4 flowing at 0.6 ml/min at 35°C.

Statistical analysis

IBM SPSS Statistics 20.0 was used for statistical analysis. Measurement data were presented as means ±S.D. (standard deviation). One-way analysis of variance was used to compare the means among the groups. Dunnett's test was employed to compare means between experimental groups and control group. $P<0.05$ was considered statistically significant.

RESULTS

Basic physical and chemical properties of GLP5

After separation with Sephadex G-100 column, seven fractions were eluted between 0.05 M NaCl and 0.55 M NaCl. Using sulfuric acid-phenol method, the sugar content of the 5th fraction (GLP5) eluted at 0.15 M NaCl was determined to be 50.5% of all the extracts. HPGPC-MALLS analysis showed that GLP5 was eluted from 22 min to 30 min as a single peak followed by a salt peak (Fig. 1). The relative molecular mass was 20700 Da with a radius of 45.5 nm and Mw/Mn ratio of 1.12, suggesting that GLP5 was a relatively uniform polysaccharide.

NMR spectrum analysis

According to ^{1}H- and ^{13}C-NMR spectrums, the chemical shifts of anomeric hydrogen were δ 4.4 and δ 4.7, the chemical shifts of the anomeric carbon were δ 102.5, suggesting that GLP5 is mainly glucopyranose connected by β-glycosidic bonds. Based on ^{1}H-^{1}H COSY spectrum and ^{1}H-^{13}C HSQC spectrum, GLP5 was inferred as mainly β-(1→3) and β-(1→6) linked glucan with three residue fragments: A, β-(1→6)-Glc*p*, B, β-(1→3)-Glc*p*, and C, β-T-Glc*p*. The chemical shifts of the 3 residues are summarized in Table 1. The coupling signals of anomeric hydrogen and anomeric carbon in the ^{1}H-^{13}C HSQC spectrum for the three fragments were A: C1-H1 (101.96/4.61), B: C1-H1 (102.93/4.78), C: C1-H1 (102.76/4.32). The additional coupling signals for remaining 5 hydrogen carbons were C2-H2 (72.83/3.22), C3-H3 (75.75/3.51), C4-H4 (69.94/3.41), C5-H5 (71.9/3.799), and C6-H6 (68.98/4.23) in the fragment A, C2-H2 (72.75/3.24), C3-H3 (84.49/3.55), C4-H4 (69.94/3.21), C5-H5 (71.2/3.53) and C6-H6 (62.71/3.53) in the fragment B and C2-H2 (71.86/3.32), C3-H3 (75.62/3.75), C4-H4 (69.64/3.33), C5-H5 (71.5/3.52), and C6-H6 (60.71/3.93) in the fragment C. These NMR data suggested that GLP5 is a β-(1→3) and β-(1→6) linked glucan.

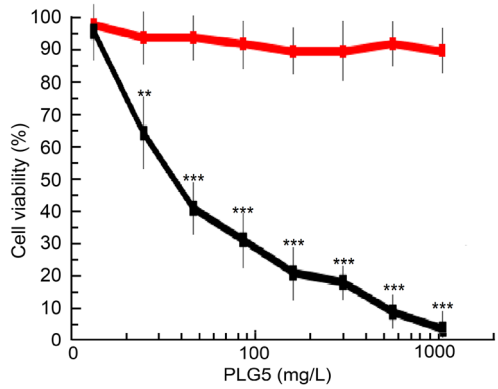

Figure 2. Dose-response curve for determining the IC 50 value for GLP5 against Jurkat (black line) and HaCat (red line) cells after exposed to GLP5 for 48 h using the MTT assays.
Values represent the mean of at least three independent experiments. ** and *** indicate $p \leq 0.01$ and ≤ 0.001 compared with the control (one-way ANOVA followed by the Dunnett post-test).

GLP5 has potent cytotoxicity to Jurkat cells, but is not toxic to HaCat cells

Among the seven fractions that were obtained from the water-soluble *G. lucidum* extracts, pilot experiments showed that GLP5 was most toxic to the Jurkat cells at concentrations less than 100 mg/l, while the other fractions did not show antiproliferative activity below 100 mg/L and were not further studied. GLP5 was assessed for cytotoxicity against Jurkat and HaCat cells using MTT assay. 1×10^{6} cells/ml were treated with various concentrations (0 to 1000 mg/l) for 48 h and cell viability was then determined. As shown in Fig. 2, the viability of Jurkat cells reduced after exposed to GLP5 in a concentration-dependent manner from over 95% at 0 mg/l to less than 5% at 600 mg/l. The half maximal inhibitory concentration (IC_{50}) was calculated to be 34.5±4.5 mg/l (or 1.7±0.21μM). On the other hand, over the same concentration range, the viability of HaCat cells was reduced from 98% to 90% after of GLP5 treatment, with an estimated IC_{50} of over 1000 mg/l.

GLP5 induces apoptosis in Jurkat cells, but not in HaCat cells

To elucidate the mechanisms underlying the cytotoxicity, we assessed the apoptosis in the HaCat and Jurkat cells after being exposed to 25 and 50 mg/l GLP5 using flow cytometry. The detection of surface exposed phosphatidyl-serine (PS) by AnnexinV-FITC has been shown to be a marker of apoptosis and we used AnnexinV-

Table 1. ^{1}H- and ^{13}C-NMR chemical shifts of GLP5 in D2O at 295K

Fragments	Glycosyl residuals	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6
A	β-1,6-Glc*p*	101.96/4.61	72.83/3.22	75.75/3.51	69.94/3.41	71.9/3.79	68.98/4.23
B	β-1,3-Glc*p*	102.93/4.78	72.75/3.24	84.49/3.55	69.94/3.21	71.2/3.53	62.71/3.53
C	β-T-Glc*p*	102.76/4.32	71.86/3.32	75.62/3.75	69.64/3.33	71.5/3.52	60.71/3.93

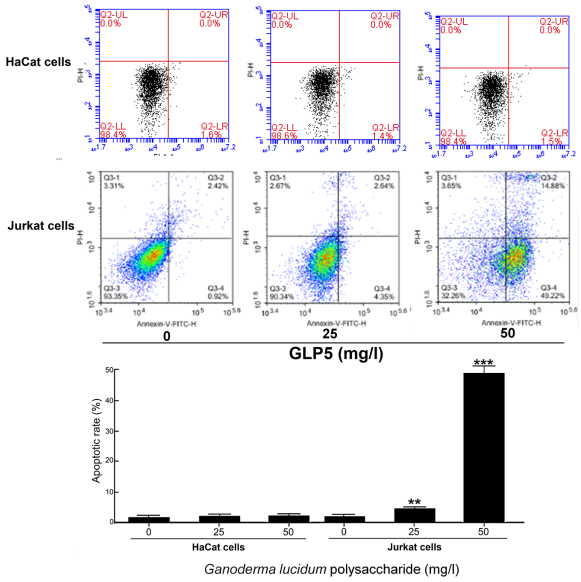

Figure 3. Flow cytometry detection of apoptosis in the Jurkat cells after exposed to GLP5.
Upper panel: Flow cytometry results. The cells were treated with 0, 25 and 50 mg/L GLP5 for 48 h and double stained with Annexin V/PI. Lower panel: apoptotic rate of early apoptosis (lower-right quadrant) from three independent experiments. ** and *** indicate $p \leq 0.01$ and ≤ 0.001 compared with the control (one-way ANOVA followed by the Dunnett post-test).

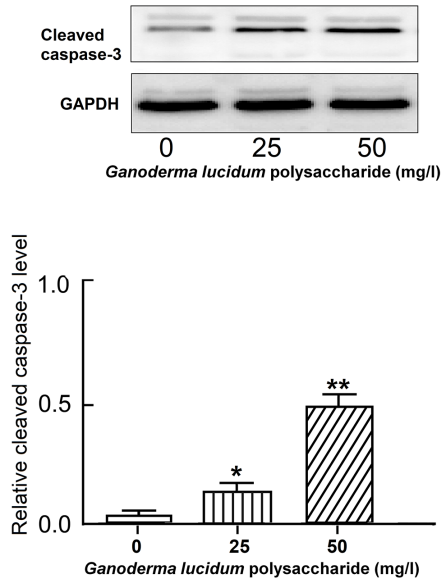

Figure 4. Expression level of cleaved caspase-3 protein in the Jurkat cells after exposed to GLP5.
Upper panel: representative Western blots following the treatment of the cells with 0, 25 and 50 mg/L GLP5 for 48 h. Lower panel: the intensities of cleaved caspase-3 relative to GAPDH from three independent experiments. * and ** indicate $p \leq 0.05$ and ≤ 0.01 compared with the control (one-way ANOVA followed by the Dunnett post-test).

FITC to stain the cells and the results showed that the early apoptotic rate increased from 0.92% in control to 4.35% and 49.22% after exposure to 25 and 50 mg/L GLP5 (Fig. 3), indicating that GLP5 could cause Jurkat cells to die through apoptosis. On the other hand, the apoptotic rate remained barely changed near 1% in HaCat cells after being exposed from 0 to 50 mg/L GLP5 (Fig. 3).

GLP5 upregulates caspase-3 and Bax expression and downregulates Bcl2 expression

We then assessed the expression of caspase-3, a key enzyme involved in apoptosis in Jurkat cells after being exposed to GLP5 using Western blot analysis. The results showed that the expression of cleaved capase-3 was significantly upregulated after Jurkat cells were exposed to GLP5 in a dose dose-dependent manner (Fig. 4), indicating that GLP5 could activate caspase-3 and apoptosis signaling pathways. Since the induced apoptosis is likely to change the expression of Bcl2 family proteins, the expressions of Bcl2 (anti-apoptotic) and BAX (pro-apoptotic) after treatment with GLP5 were also assessed using Western blot analysis. Bcl2 expression was reduced significantly after the cells were exposed to 25 mg/L GLP5 after 24 h and was dramatically reduced after the cells were exposed to 50 mg/L GLP5 (Fig. 5). At the same time, BAX expression was significantly increased upon treatment with GLP5 (Fig. 5), particularly upon treatment with 50 mg/L GLP5.

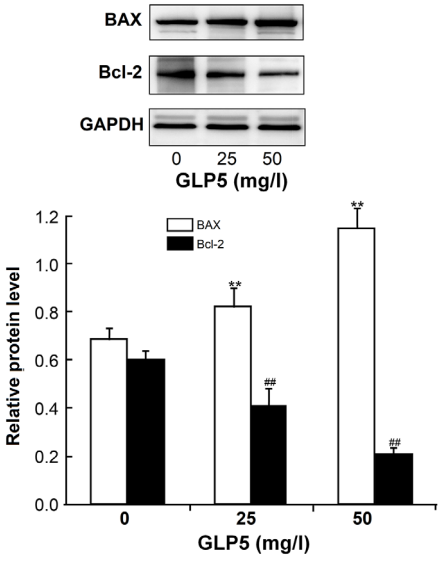

Figure 5. Expression level of BAX and Bcl2 in the Jurkat cells after exposed to GLP5.
Upper panel: representative Western blots following the treatment of the cells with 0, 25 and 50 mg/L GLP5 for 48 h. Lower panel: the intensities of BAX and Bcl2 relative to GAPDH from three independent experiments. ** and ## indicate $p \leq 0.01$ compared with the control for BAX and Bcl-2, respectively, (one-way ANOVA followed by the Dunnett post-test).

DISCUSSION

Clinically, leukemia is mainly treated with chemotherapy that uses multiple drugs, but the overall prognosis has not been satisfactory due to drug tolerance and serious adverse reactions. Polysaccharides are natural polymers and some of them have been demonstrated to have antitumor activity with less adverse reactions and are considered to be excellent drug candidates in various tumors (Bian *et al.*, 2020; Sadreddini *et al.*, 2017; Wang *et al.*, 2019), although their activity on leukemia is relatively less known. In this study, the cytotoxicity of GLP5, a

polysaccharide extracted from *G. lucidum* was tested on leukemia cells. The results showed that GLP5 has potent cytotoxicity against Jurkat cells with a IC_{50} of 34.5±4.5 mg/L and could activate apoptosis pathways, leading to increased apoptosis and upregulation of pro-apoptotic genes. Therefore, GLP5 may be further investigated as a potential therapeutic agent for leukemia.

A variety of polysaccharides have been found to have inhibitory effect on leukemia cells. Polysaccharide extracted from the fruits of *Lycium barbarum* was found to be able to enhance the activity of NK cells from leukemia patients against HL-60 cells by upregulating NKG2D ligand MICA (Xiao & Wu, 2017) and lentinan, a polysaccharide prepared from the shiitake mushroom (*Lentula edodes*), was shown to inhibit the proliferation of HL-60 cells in a concentration-dependent manner. It also triggered the expression of cleaved PARP, cleaved caspase-9, and cleaved caspase-3 and the release of cytochrome c into the cytoplasm (Ma *et al*., 2019). In addition, polysaccharides from *Cistanche deserticola* (Zhang *et al*., 2016), *Agaricus blazei* (Li *et al*., 2013), and *Pleurotus eryngii* (Chen *et al*., 2015) also exhibit cytotoxicity against various leukemia lines. Recently, glycosaminoglycan-like polysaccharides extracted from the common cockle (*Cerastoderma edule*) have been shown to have antiproliferative activity on chronic myelogenous leukemia and relapsed acute lymphoblastic leukemia cell lines (Aldairi *et al*., 2018). In this study, GLP5 was found to be a β-(1→3) and β-(1→6) linked glucan based on HPGPC-MALLS and NMR analyses. We used MTT assays to assess the cytotoxicity of GLP5 against the Jurkat cells and found that GLP5 is highly toxic to the Jurkat cells. The IC_{50} is similar to that of lentinan against human lymphocytes (Peter *et al*., 1988). Subsequent apoptosis assays based on flow cytometry showed that apoptosis was induced after the Jurkat cells were exposed to GLP5, suggesting that at least one of the mechanisms underlying the toxicity is the activation of apoptosis pathways. This is consistent with the results obtained in lentinan on HL-60 cells (Ma *et al*., 2019). On other hand, when GLP5 was assessed against non-cancerous HaCat cells, no cytotoxicity and apoptosis were observed in the tested concentration ranges, suggesting that GLP5 is selectively toxic to leukemia cells. The reason for this selectivity is unclear. It might be due to the differences between these cells in cell surface ligands/receptors, permissibility to the GLP5 and targets in the cells.

Apoptosis, also known as programmed cell death, is one of the most important pathways leading to cell death after cancer is treated with chemotherapy (Johnstone *et al*., 2002). Induction of defective apoptosis in cancer is one of the major causative approaches for cancer treatment (Goldar *et al*., 2015; Melet *et al*., 2008) and apoptosis is considered as a major target for cancer therapy with pro-apoptotic agents (Carneiro & El-Deiry, 2020). To further confirm that GLP5 induced apoptosis in Jurkat cells, we analyzed the expression of several key genes involved in apoptosis pathways and found that pro-apoptotic genes that cleaved caspase-3 and BAX were upregulated and anti-apoptotic gene Bcl-2 was down-regulated after the Jurkat cells were exposed to GLP5, indicating that GLP5 has an impact on apoptosis pathways. These results are consistent with the previous studies with lentinan on HL-60 cells (Ma *et al*., 2012), *Sargassum fusiforme* polysaccharide on human erythroleukemia cells (Ding *et al*., 2020) and pectin-like polysaccharide from *Polygala tenuifolia* on pancreatic cancer cells (Bian *et al*., 2020). Caspase-3 is the best recognized biochemical hallmark of both early and late stages of apoptosis and exists in the form of pro-caspase-3. When apoptosis is induced by a wide variety of apoptotic signals, caspase-3 is hydrolyzed to cleaved caspase-3, resulting in proteolysis and apoptosis (Choudhary *et al*., 2015; Crowley & Waterhouse, 2016). Therefore, detection of cleaved caspase-3 is considered as a reliable marker for cells that are dying, or that have died by apoptosis (Crowley & Waterhouse, 2016). After incubation with GLP5, the level of cleaved caspase-3 increased as the concentration of GLP5 increases, indicating that GLP5 could induce apoptosis in the Jurkat cells.

In addition, the BCL-2-associated X apoptosis regulator (BAX), is a pro-apoptotic regulator that is involved in a wide variety of cellular activities. This protein increases the opening of the mitochondrial voltage-dependent anion channel (VDAC), leading to the loss of membrane integrity, the release of cytochrome *c*, and cell death (Kuwana *et al*., 2020; Maes *et al*., 2019). Apoptosis induced by various anticancer agents often results in upregulated BAX and downregulated Bcl2 expressions. For example, after being treated with mangiferin, BAX and Bcl2 levels were changed, leading to apoptosis in the CNE2 nasopharyngeal carcinoma cells (Pan *et al*., 2014), and upregulation of BAX and downregulation of Bcl2 also result in apoptosis induced in various human cancer cell lines by 3-NC (2-amino-4-(3-nitrophenyl)-3-cyano-7-(dimethylamino)-4H-chromene) (Naseri *et al*., 2015). Altered ratio of proapoptotic and anti-apoptotic Bcl2 family members Bax and Bcl2 after exposure to GLP5 is likely a key driver leading to apoptosis. However, how GLP5 induces the changes of expression of these apoptosis-related genes needs to be further elucidated. In human colon cancer, polysaccharide hydrolysate derived from *G. lucidum* could induce apoptosis to suppress the growth of cells by upregulating Bax, phospho-extracellular regulated protein kinases (P-ERK), and cleaved caspase-3 and downregulating Bcl-2, phospho-serine/threonine kinase 1 (p-Akt1), and cyclo-oxygenase-2 (COX-2) (Bai *et al*., 2020). On other hand, polysaccharide extracted from *G. lucidum* may enhance the activity of NK cells to activate DNAX-associated protein 10/phosphoinositide 3-kinase/extracellular regulated protein kinases to mediate its cytotoxicity (Yang. *et al*., 2019). It can also bind with NK cells to act on several immune receptors including Dectin-1, complement receptor (CR3), and TLR-2/6, then trigger both innate and adaptive response and enhance opsonic and non-opsonic phagocytosis to exert antitumor activity (Xiao *et al*., 2020).

Although GLP5 is demonstrated to have potent inhibitory activity against the Jurkat cells and to be a single β-glucan, further studies with more cancer and non-cancerous cell lines are needed to define its in vitro activity and selectivity against cancer. It may be further purified to avoid activity from other compounds to better define its potency. Physical and chemical modifications such as phosphorylation (Hu *et al*., 2020), microwave irradiation (el Knidri *et al*., 2018), acetylation, and carboxymethylation (Ma *et al*., 2012) may be used to generate more potent derivatives with increased solubility and stability for screening drug candidates from GLP5.

CONCLUSION

Our experimental data indicate that GLP5 is a β-(1→3) and β-(1→6) linked glucan, it induces apoptosis and inhibits the proliferation of Jurkat cells but not of HaCat cells. The activity is likely mediated through the upregulation of pro-apoptotic genes and downregulation

of anti-apoptotic genes. Thus, this new polysaccharide and its derivatives may be further assessed for selectivity and safety against leukemic cells and normal cells for potential therapeutic use in leukoma and other cancers.

Conflict of interest

None.

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