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Sesamin Acts as Anti-leukemic Compound Interacting with Novel Phosphoprotein Targets and Inducing Apoptosis in Leukemic Cells

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ABSTRACT

Leukemia is one of the high-incidence cancers that is characterized by an abnormal production of immature white blood cells. Subject to many reports on the side effects of conventional chemotherapy, herbs and natural compounds have been studied as an alternative medicine. In this study, sesamin, a lignan in sesame seed with pharmaceutical functions including anti-cancer, was chosen and treated with MOLT-4 and NB4 leukemic cell lines in various concentrations for 24 and 48 hours. The effect of sesamin on cell inhibition and expression levels of apoptotic genes in leukemic cell lines were investigated by MTT assay and real-time PCR, respectively. Moreover, apoptotic proteins were studied by mass spectrometry and bioinformatics tools to investigate the relation between sesamin and targeted proteins. Results showed that sesamin increased cell inhibition in both cell lines in dose- and time-dependent manner. Levels of caspase-3, -7, -8, and -9 gene expressions significantly increased, while BCL-2 decreased drastically in sesamin-treated cells. From bioinformatics study, PARP4, IPPK and caspase family proteins were found to be involved in sesamin that induced apoptosis in leukemic cells. Besides, doxorubicin, a chemotherapeutic drug, also shared the same protein targets as sesamin in apoptosis pathway. Sesamin demonstrates its potential to enhance cell inhibition and promotes cell apoptosis in both MOLT-4 and NB4 leukemic cell lines. This study will benefit the development of sesamin as an effective anti-leukemia drug in the future.

Keywords: Sesamin, leukemia, MOLT-4, NB4, apoptosis, Doxorubicin

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Introduction

Sesamin is a major lignan extracted from sesame seeds, an economically important oil seed crop known as *Sesamum indicum*, which has recently been considered as a healthy food supplement worldwide (1, 2). It has been reported to have various pharmaceutical properties. Recent studies have shown that sesamin has anti-oxidation (3), anti-inflammation (4), anti-hypertension (5), and anti-cancer activities (6).

Sesamin possesses an ability to act as a pro-antioxidant when metabolized to antioxidant compounds, namely, mono- and di-catechol metabolites, in liver cells by hepatic enzyme that could eliminate reactive oxygen species (ROS) (7). It also exhibits the anti-inflammation activity by protecting the cells from oxidative stress (8). Moreover, sesamin was demonstrated for being effective as a treatment of hypertension and cardiovascular diseases after the administration of sesamin in both cell line and animal models (9). It was also reported to prevent hypertension by inhibiting the enzyme cytochrome P_{450} family CYP4F2 and 20-hydroxyeicosatetraenoic acid (20-HETE) that play a role in hypertension pathogenesis (10). Interestingly, sesamin also demonstrated an anti-cancer activity in distinct pathways such as cell cycle arrest promotion, transcription factors inhibition, and apoptosis induction (6, 11-14). There were studies that exhibited the proliferation of various cancer cell types was suppressed by sesamin treatment including lung cancer, myeloma, prostate cancer, colon cancer, pancreas cancer, breast cancer, and leukemia (15). However, there is less information according to sesamin effect on leukemia, one of the top ten cancer incidences during 2016-2018. The estimated number in Thailand based on nationwide data collection is 5.3 cases per 100,000 population in males and 4.5 cases per 100,000 populations in females (16).

Leukemia, especially acute leukemia, is reported to have more severity than chronic leukemia because of its rapid progression. Patients with acute leukemia need to be diagnosed and received a treatment as soon as possible as it develops very quickly. It could increase the risk of having frequent infections, fatigue, nausea and vomiting, anemia, bleeding, and etc. (17, 18). These complications can cause death. The estimated 1,580 deaths from ALL and 11,400 deaths from AML in 2021 in the United States (19). Treatment may include a chemotherapy which could give high side effects to leukemic patients (20). Using natural compounds or herbs is considering as an alternative cancer treatment given to less side effects comparing to the conventional chemotherapy. Nonetheless, the underlying mechanisms behind sesamin-induced inhibition of the proliferation and the induction of apoptosis in cancer cells are still unclear and require further studies. In this study, we investigated the effect of sesamin on the induction of apoptosis in leukemic cell lines through apoptotic signaling pathway.

Materials and Methods

Compound and chemical reagents

Sesamin (Sigma Aldrich, USA) was used as a treated cell reagent. Dimethyl sulfoxide (DMSO) was used to dissolve sesamin. MTT dye solution 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide used in MTT assay was from Sigma Aldrich, USA; RevertAid First Strand cDNA Synthesis Kit from Thermo Scientific, USA; iQTM SYBR® Green Supermix from Bio-RAD, Canada.

Leukemic Cell culture

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Acute lymphoblastic leukemia (ALL) MOLT-4 and acute promyelocytic leukemia (AML) NB4 cells purchased from Cell Lines Service GmbH (Eppelheim, Germany) were cultured in 10% fetal bovine serum and 2% penicillin-streptomycin supplements in RPMI-1640 medium at the condition of 37 ^{o}C and 5% CO_2. The cultured medium was changed every 3 days.

Cell inhibition assay

Sesamin at different concentrations 0, 50, 100, and 200 µg/mL were treated in $1x10^4$ cells/mL leukemic cell lines in 96-well plate and incubated for 24 and 48 hours at 37^{o}C with 5% CO_2. Leukemic cells in medium without sesamin were used as an internal control. The MTT assay, a colorimetric assay for measuring mitochondrial dehydrogenase activity in viable cells, was performed to determine leukemic cell inhibition activity. As this enzyme reduces the yellow tetrazolium salt, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) into the purple formazan dyes, the intensity of purple color could reflect the number of living cells in each well. After incubation, 50 µL of 1 mg/mL MTT dye solution was added into each well and kept incubated for 4 hours in 5 % CO_2 at 37^{o}C. Then, 100 µL of solubilized solution (10% Sodium dodecyl sulfate (SDS) in 0.01 M HCl) was added into each well. The plates were incubated overnight in 5% CO_2 at 37^{o}C. Then, the spectrophotometer was used to measure the 570 nm absorbance. The percentage of cell inhibition of both MOLT-4 and NB4 in different doses and times were calculated. The inhibitory concentration (IC_{50}) of both MOLT-4 and NB4 was determined by linear regression analysis. Doxorubicin was reported as a positive control with cytotoxicity in MOLT4 and NB4 at 90% and 88.2% of cell inhibition percentage, respectively. While IC_{50} of doxorubicin was also reported at concentration of 0.1 µM in MOLT4 and 0.4 µM in NB4 after 24 hour-treatment (21).

In addition, peripheral blood mononuclear cells (PBMC) were extracted by Ficoll-Hypaque separation. Cells were treated with sesamin at various concentrations 0, 50, 100, and 200 µg/mL, and incubated for 48 hours.

Apoptotic gene expression

Real-time PCR assay had been used to determine the gene expression levels of apoptotic signaling pathway including *caspase -3, -7, -8, -9,* and *BCL-2* genes. The leukemic cell lines with 5 x 10^5 cells/mL concentration were treated with sesamin at their IC_{50} values (100 µg/mL sesamin in MOLT-4 and 120 µg/mL sesamin in NB4), in a 24-well plate and incubated for 48 hours. Untreated cells were used as an internal control. RNA was extracted from the cell by TRIzol®Reagent and measured the concentration by NanoDrop spectrophotometer at 260/280 nm. Then, RNA was converted into cDNA by RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) following the manufacturer's protocol. The cDNA was amplified by real-time PCR with *caspase -3, -7, -8, -9,* and *BCL-2* primers with distinct conditions (Table 1) using iQ™ SYBR® Green Supermix (Bio-RAD, Canada) as a DNA binding dye. Real-time PCR was performed on iCycleriQ® Multicolor Real-time PCR Detection system under certain conditions. The products were normalized and quantitated the amount of gene expression.

Phosphoprotein and Bioinformatics analysis

Phosphoproteins of sesamin treated MOLT-4 and NB4 cells as well as untreated cells were analyzed by liquid chromatography in line with tandem MS mass spectrometry (LC-MS/MS). In brief, after treated sesamin in both leukemic cell lines in each time cost, cells were collected. Protein concentration of each

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sample was measured by Lowry protein assay using bovine serum albumin (BSA, Thermo Scientific, USA) as a standard. The immobilized metal affinity column (IMAC) obtained from Pierce Phosphoprotein Enrichment Kit (Thermo Scientific) and Protein Desalting Spin Column Kit from Thermo Scientific was used for enriching phosphoproteins and removing salt from samples, respectively. Lowry assay was performed again to measure protein concentration of desalted samples. Then, phosphoproteins were reduced, alkylated, and digested into phosphopeptides. Phosphopeptide samples were injected into mass spectrometer (Waters Corp., Manchester, UK)

Table 1. A list of apoptotic gene primers.

Primers	Sequence (5'→3')	Length (bp)	Condition (39 cycles)
Caspase-3	F: TTCAGAGGGGATCGTTGTAGAAGTC R: CAAGCTTGTCGGCATACTGTTTCAG	25 25	95ºC, 10 s 68ºC, 10 s 72ºC, 40 s
Caspase-7	F: CCAATAAAGGATTTGACAGCC R: GCATCTGTGTCATTGATGGG	21 20	95ºC, 10 s 65ºC, 10 s 72ºC, 40 s
Caspase-8	F: GATCAAGCCCCACGATGAC R: CCTGTCCATCAGTGCCATAG	19 20	95ºC, 10 s 65ºC, 10 s 72ºC, 40 s
Caspase-9	F: CATTTCATGGTGGAGGTGAAG R: GGGAACTGCAGGTGGCTG	21 18	95ºC, 10 s 65ºC, 10 s 72ºC, 40 s
BCL-2	F: ATGTGTGTGGAGACCGTCAA R: GCCGTACAGTTCCACAAAGG	20 20	95ºC, 10 s 65ºC, 10 s 72ºC, 40 s
GAPDH	F: GCACCGTCAAGGCTGAGAA R: AGGTCCACCACTGACACGTTG	19 21	95ºC, 10 s 60ºC, 10 s 72ºC, 40 s

The data from LC-MS/MS was first analyzed by DeCyder MS Differential Analysis software for protein quantitation based on the intensity of each MS signal. Next, protein identification was analyzed by Mascot software (Matrix Science, London, UK) searching against Homo sapiens protein using UniprotKB database. Then, PANTHER database along with UniprotKB database were used for categorizing proteins into different types, for example, biological regulation, metabolic process, immune system process, or apoptotic process, etc. (22). The protein quantitation was conducted using heat map visualization from Multi-Experiment Viewer (MeV) software (23). In addition, the possibility of the interaction between apoptotic phosphoproteins and sesamin was studied by using STITCH database which is a bioinformatics tool to study the relation between protein-protein and protein-chemical interaction (24).

Statistical analysis

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The triplicated experiments were performed where each effect result was compared against the control (untreated cell) and resulted as a mean ± SEM. The statistical analysis was conducted by student t-test using SPSS statistical program (SPSS Inc., Chicago, IL, USA) with statistically significant differences at *, *p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001*.

Results

Effect of sesamin on leukemic cell inhibition

MTT assay was used to measure the percentage of cell inhibition of sesamin treated leukemic cells. Following sesamin treatment at 24 hours and 48 hours, the results of cell inhibition showed significantly increasing in both MOLT-4 and NB4 leukemic cell lines with dose- and time-dependent manner. The IC_{50} at 48 hours of incubation for MOLT-4 was 104.84 µg/ml whereas NB4 had higher value at 121.00 µg/ml. Peripheral blood mononuclear cells (PBMC) were extracted by Ficoll-Hypaque separation as normal cells and treated with sesamin at various concentrations 0, 50, 100, and 200 µg/ml, following 48 hours incubation. Untreated cells (medium without sesamin) were assigned as an internal control. Then, the activity of cell inhibition was measured by MTT assay where the results showed no significant increase of PBMC's cell inhibition after sesame treatment (Figure 1).

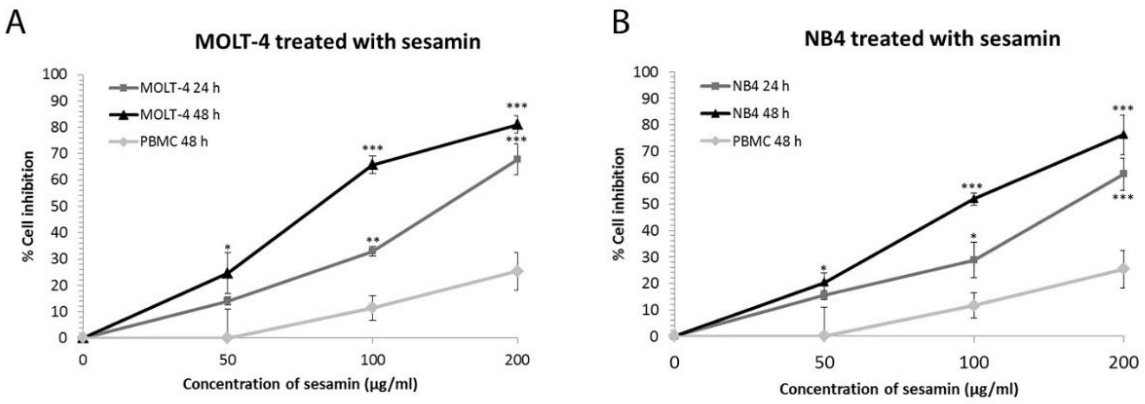


Fig. 1. Cytotoxic effects of sesamin on (a) MOLT-4 and (b) NB4 cell line after 24 hour and 48 hour treatments compare with PBMC cells after 48 hour treatment. The results showed the percentage of cell inhibition measured by MTT assay. IC_{50} at 48 hours for MOLT-4 and NB4 were reported as 104.84 and 121.00 µg/mL, respectively. Data were expressed as the mean±SD of independently three experiments. The differences between sesamin treated and untreated were examined for the statistical significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Effect of sesamin on apoptotic gene expression

MOLT-4 and NB4 leukemic cell lines were treated with sesamin for 48 hours at their IC_{50} concentration of 100 and 120 µg/mL, respectively. Untreated cells were used as an internal control. Gene expression was analyzed using real time quantitative PCR assay. The results showed that pro-apoptotic gene expression of *caspase-3*, *-7*, *-8*, and *-9*, were significantly up regulated whereas *BCL-2*, an anti-apoptotic gene, was adversely down regulated in both MOLT-4 and NB4 cell lines (Figure 2).

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Effect of sesamin on phosphoproteins profile in MOLT-4 and NB4

Phosphoprotein profile of sesamin treated cells and untreated cells in MOLT-4 and NB4 were identified by LC-MS/MS. Number of phosphoproteins found in both MOLT-4 and NB4 after sesamin treatment were reported to be 79 in total. Using the criteria of presenting in sesamin treated cells but not in untreated, showed sets of 14 phosphoproteins presented in MOLT-4 treated cells and 14 phosphoproteins presented in NB4 treated cells in Venn diagram (Figure 3). PANTHER and UniprotKB database were used to categorize the biological process of these phosphoproteins (Tables 2, 3).

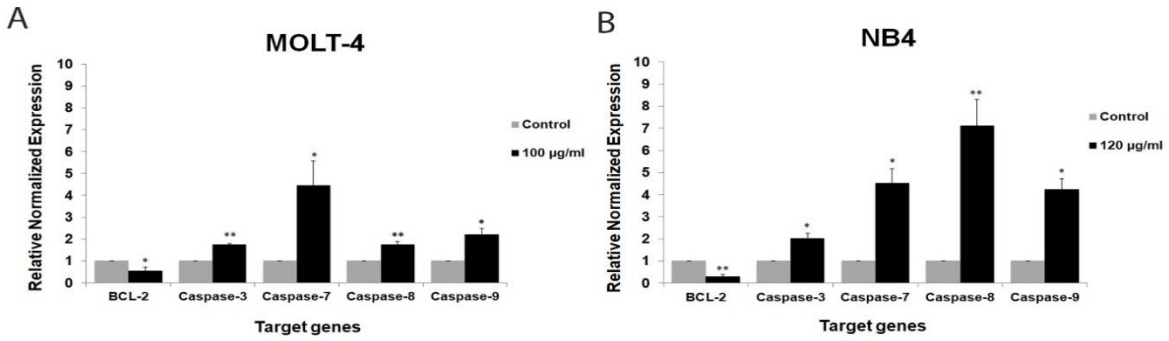


Fig. 2. The relative normalized expression of apoptotic genes in (a) MOLT-4 and (b) NB4 after sesamin treatment at IC_{50} values of 100 and 120 µg/mL, respectively, for 48 hours. The experiment was determined by Real-time PCR. Data were expressed as the mean±SEM of three independent experiments. The differences between sesamin treated and untreated were examined for the statistical significance: *, $p < 0.05$; **, $p < 0.01$.

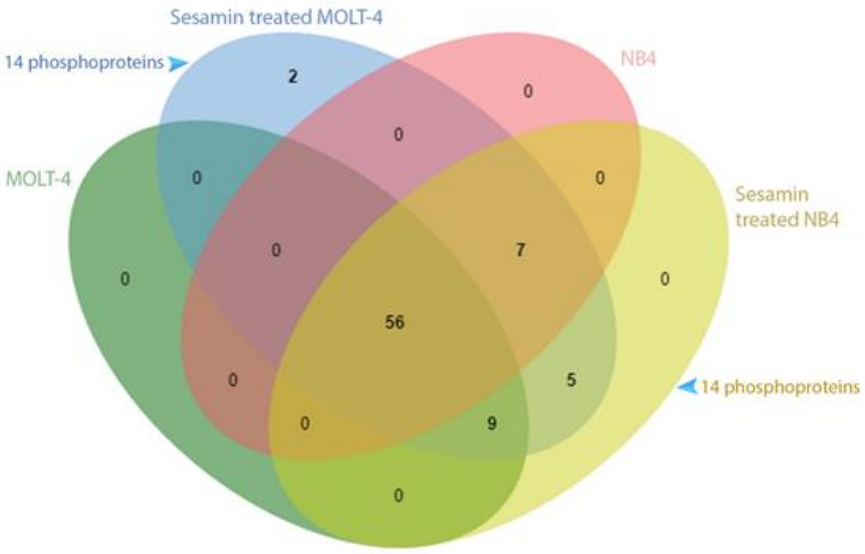


Fig. 3. Venn diagram shows a set of 14 phosphoproteins present only in sesamin treated MOLT-4 (blue) and a different set of 14 phosphoproteins present only in sesamin treated NB4 (yellow).

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Table 2. A list of 14 phosphoproteins with their biological functions expressed only in MOLT-4 treated with sesamin analyzed from PANTHER and UniprotKB database.

Protein name	Accession number	Biological function
Phenylalanine-4-hydroxylase	gi|4557819	Enzyme regulation
Interleukin-1 receptor type 1 isoform X7	gi|530368626	Mediates interleukin-1-dependent activation of NF-kappa-B, MAPK and other pathways.
S67384_1 preprogastrin-releasing peptide, partial	gi|4261690	Unknown
Family with sequence similarity 86, member C, isoform CRA_h	gi|119595208	Unknown
hCG2041389	gi|119604326	Unknown
PARP4 protein	gi|118142848	Cell death
VIP36-like protein isoform X1	gi|578804985	Regulation of export from the ER of glycoproteins
Unnamed protein product	gi|158255130	Unknown
CLIP-associating protein 2 isoform X25	gi|578805860	Regulation of Cell cycle/Cell division
Chain B, Crystal Structure Of The Kix Domain Of Human Recql5	gi|512125382	DNA replication, transcription and repair
hCG2041708	gi|119632065	Unknown
Chain A, Nmr Structure Of The C-Terminal Pas Domain Of Hif2a	gi|159162817	Transcription regulation
Nuclear antigen H731	gi|1825562	Programmed cell death 4
Unnamed protein product	gi|40039962	Unknown

Table 3. A list of 14 phosphoproteins with their biological functions expressed only in NB4 treated with sesamin analyzed from PANTHER and UniprotKB database.

Protein name	Accession number	Biological function
hCG2036718	gi|119571777	Unknown
Zinc finger protein 622	gi|15529978	Positive regulation of apoptotic process
Zinc finger protein 711 isoform X5	gi|578838469	Transcription regulator required for brain development
Lysine-specific demethylase 4A isoform X3	gi|530363648	Histone demethylation
Unnamed protein product	gi|40043974	Unknown
Unnamed protein product	gi|40980138	Unknown
Syntaxin binding protein 1 variant	gi|62087940	Regulation of synaptic vesicle docking/fusion
Inositol-pentakisphosphate 2-kinase	gi|12232423	inositol phosphate metabolic process
Basic proline-rich protein-like	gi|578805478	Unknown
hCG2041389	gi|119604326	Unknown
Phenylalanine-4-hydroxylase	gi|4557819	Enzyme regulation
Family with sequence similarity 86, member C, isoform CRA_h	gi|119595208	Unknown
CLIP-associating protein 2 isoform X25	gi|578805860	Regulation of Cell cycle/Cell division
Unnamed protein product	gi|40039962	Unknown

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These individual phosphoproteins were further analyzed for the quantitative expression in various times after sesamin treatment using heatmap visualization from MeV software (Figure 4). The result showed that several phosphoproteins were upregulated in comparison with the untreated sample. Among the upregulated phosphoproteins, there were some proteins involved in cell death pathway such as PARP4 in MOLT-4 or inositol pentakisphosphate-2-kinase (IPPK) in NB4.

The interaction between apoptotic proteins and chemotherapeutic drug

To study the involvement between caspase family proteins, selected phosphoproteins and anti-cancer drugs, bioinformatics analysis was performed. The results showed the interaction observed between the BCL-2, caspase family proteins, PARP4, IPPK, and the doxorubicin, a cancer chemotherapy drug (Figure 5).

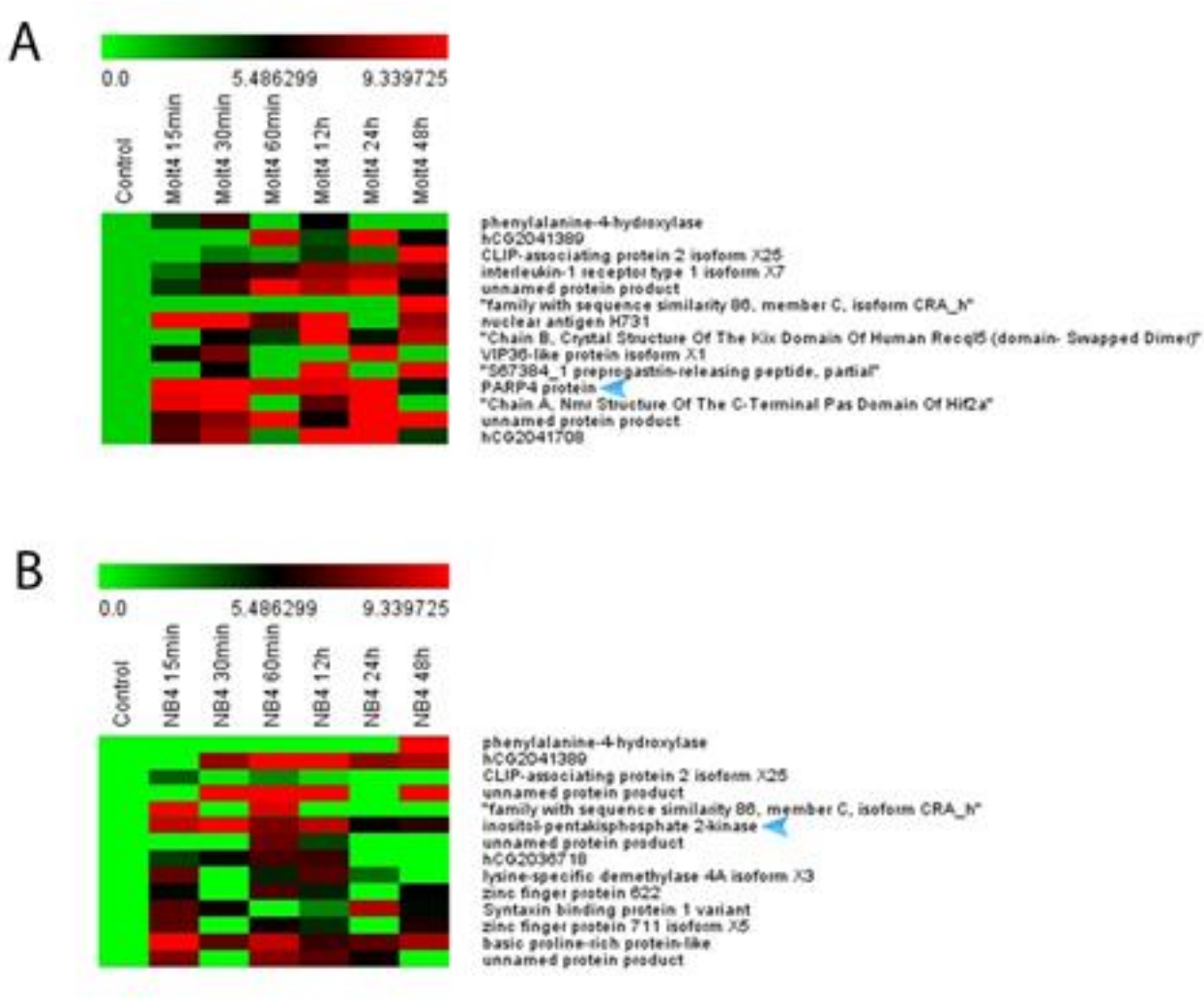


Fig. 4. Heatmap of phosphoproteins with significant difference in expression of (A) MOLT-4 and (B) NB4 treated with sesamin in various times compare with the control. The color indicates the expression level from low (green) to high (red). The blue arrow indicates the selected phosphoproteins.

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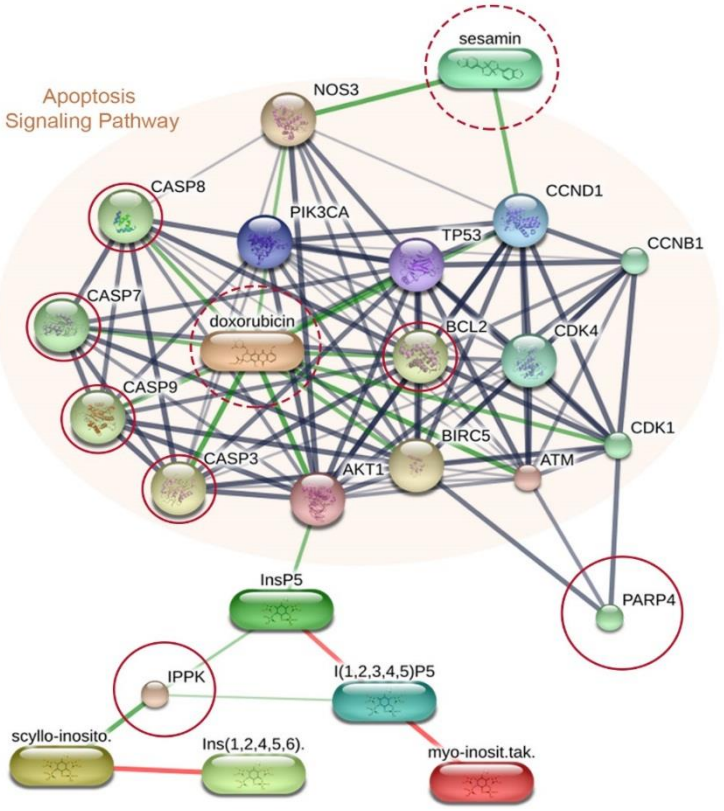


Fig. 5. The schematic diagram shows the interactions between sesamin, caspase-3 (CASP3), caspase-7 (CASP7), caspase-8 (CASP8), caspase-9 (CASP9), BCL-2, and selected phosphoprotein; PARP4 and IPPK, in MOLT-4 and NB4 leukemic cell lines using STITCH database. The round shape represents protein. The oval shape represents chemical. Red circle indicates selective proteins. Red dashed circle indicates selective chemicals.

Discussion

Apoptosis is a programmed cell death (PCD) which plays a role in regulating the homeostasis of hematopoietic system by keeping the equilibrium between cell proliferation and cell death. Accordingly, the apoptosis reduction or growing proliferation may lead to an event of cancer (25). Then, the enhancement of apoptosis level which conceivably executed by various therapies is one of a mean to treat cancer. Conventional chemotherapy has been used for cancer treatment. However, it causes adverse effects to the patients (20). Therefore, natural compounds and herbs have been recently developed as an alternative treatment and a new cancer therapeutic.

Several evidence showed numerous therapeutic activities by sesamin comprising anti-hypertensive, anti-oxidant, anti-inflammatory, and anti-cancer (1). By focusing on cancer, there are many previous studies that reported the ability of sesamin on anti-cancer in different types of cell lines. Sesamin can inhibit cell proliferation by enhancing growth arrest at G1 phase in cell cycle progression and apoptosis in breast cancer cell line (T-47D and MCF-7), transformed renal cell line (293T), immortalized keratinocyte cell line (HaCaT), melanoma cell line (UACC-62), osteosarcoma cell line (MG63), and lung cancer cell line (A549

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and H1792) (12-14). It also promotes cell cycle arrest at G2/M phase and induces apoptosis of HepG2 liver cancer cell line through STAT3 signaling pathway (11) with dose-dependent manner. Conversely, the mechanism of sesamin treated leukemic cells is still uncertain. In this study, sesamin demonstrated anti-cancer properties to MOLT-4 and NB4 leukemic cell lines.

Sesamin could enhance cell inhibition and promote cell apoptosis in both MOLT-4 and NB4 leukemic cell lines in dose- and time-dependent manner. However, there is a minimal cytotoxic effect on normal PBMC. Similarly, the report (26) also showed sesamin with less cytotoxicity in PBMCs.

Apoptosis could be triggered by many pathways. Based upon the caspase involvement, it was classified into two major pathways as caspase-independent pathway and caspase-dependent pathway. The caspase-independent pathway can occur when in the case that mitochondria membrane potential change was induced by death stimulus. There are many ligands and cell death stimuli involved in this pathway such as drug, virus infection, granzyme A, and some free radical groups. It leads to the creation of reactive oxygen species (ROS) or the release of AIF and endonuclease G factor, which translocated to nuclear for DNA cleavage and exerted apoptosis. On the other hand, the caspase-dependent pathway involves caspases participation in both intrinsic- and extrinsic-apoptotic signaling pathway. Intrinsic pathway promotes the apoptosis process through mitochondria by activating the mitochondrial outer membrane permeabilization (MOMP) and the proteins on its membrane such as BCL-2, Bax, etc. Then, cytochrome c releases and activates caspase-9 and caspase-3/7 to exert the apoptosis. Besides, the extrinsic apoptotic pathway is promoted by death ligands through caspase-8 and caspase-3/7. However, caspase-8 can also drive through a pro-apoptotic factor, called Bid, and induces the release of cytochrome c from MOMP leading to the activation of caspase-9 and caspase-3 as similar as the intrinsic pathway (27-29).

Results from this study showed that sesamin causes down regulation of *BCL-2* and up-regulation of *caspase-3*, *-7*, *-8*, and *-9* gene expression in both MOLT-4 and NB4 leukemic cell lines. *BCL-2*, as an anti-apoptotic gene located on mitochondrial membrane, prevents the release of mitochondrial apoptotic factors for example cytochrome c and AIF into the cytoplasm and thus plays an important role in inhibiting the apoptosis process. On the other hand, caspase cascade takes part in promoting all perspective of apoptosis. *Caspase-8* and *caspase-9* act as an upstream caspase coupling the apoptosis stimuli to the downstream caspases; *caspase-3* and *caspase-7*, which then triggered cell death with apoptosis morphological features. *Caspase-8* is accountable for the extrinsic pathway that initiates apoptosis by trans membrane death receptors, for example Fas receptor, TNF receptor, and TRAIL receptor. *Caspase-9* is an intrinsic pathway caspase activated from MOMP and the release of cytochrome c, which then leads to the cleavage and the activation of executioner caspases (30, 31). *Caspase-3* and *caspase-7* are the executioner caspases with a distinct function in apoptosis. *Caspase-3*, which considered as a major executioner caspase of apoptosis, is responsible for the initiation of apoptotic degradation phase, whereas *caspase-7* is engaged in cell detachment that helped remove apoptotic cells from extracellular matrix. However, working together between *caspase-3* and *caspase-7* would induce and execute the apoptosis effectively (32). Thus, the induction and the execution of apoptosis processes were regulated by *BCL-2* and caspase family genes. Ultimately, the results of this study demonstrated that sesamin treatment in MOLT-4 and NB4 were involved in caspase-dependent cascade apoptosis signaling pathway.

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Sesamin also affect the leukemic cells in protein level. Interestingly, few phosphoproteins were up-regulated in sesamin treated MOLT-4 and NB4 cells were identified to be involved in apoptosis pathway. There was poly (ADP-ribose) polymerase family member 4 (PARP4) that was upregulated from the control sample in MOLT-4 leukemic cell line. PARPs are a family of protein composed of 17 members that were associated with many cell regulations such as DNA repair, gene stability or apoptosis. There are many studies that reported PARP was found to be involved in programmed cell death (33-35). PARP had also been studied together with p53, a tumor suppressor protein, and caspase-3 in their interaction in rat models after irradiation. It found that PARP, p53, and caspase-3 expression were up regulated and highly coordinated during apoptosis process (36). In PARPs family, PARP1 is the major protein in the family that plays a crucial role in apoptosis. However, other PARP family members were also reported to have taken part in cell death (34). PARP4 is grouped in a different category from other PARPs members by their structure but it also may serve as a tumor suppressor protein like the others (37). Previous study also reported about PARP4 and its potential to play the role as a tumor suppressor when PARP4 knockdown has enhanced the cell proliferation in HCC1143 breast cancer cells (38). These data can support our study that PARP4 could be involved in apoptosis induction in sesamin treatment of leukemic cells.

While in NB4 cells, there was a high expression of inositol-pentakisphosphate 2-kinase (IPPK) upregulating the sesamin treated cell. IPPK is an enzyme that phosphorylates inositol pentakisphosphate (InsP5) at position 2 to form inositol hexakisphosphate (InsP6). There were some previous studies that showed the evidence of InsP6 activity in human cancer cell lines that InsP6 was able to reduce cell proliferation rate in both Jurkat acute lymphoblastic leukemia cells (39) and A2058 melanoma cells (40). InsP6 and Ins could enhance the anti-cancer activity and control the metastasis of cancer on *in vivo* study (41). InsP6 was also reported responsible for inhibiting the activation of phosphoinositide 3-kinase (PI3K)/Akt which plays the roles in cell survival and proliferation process and also for giving a cytotoxic effect inducing apoptosis to cancer cells (42). Interestingly, IPPK was found to have a function associated with cullin-RING ligase (CRL) regulation in various cellular activities, including CRL neddylation that plays a role as an anti-cancer target (43). These data indicated that the up regulation of IPPK could be involved in inositol metabolism pathway which was associated with apoptosis pathway leading to the cell death.

The STITCH database tool was used to demonstrate the relationship between these selective apoptotic proteins, anti-cancer drug and sesamin. The data showed the interaction of indirect linkage between sesamin, PARP4, IPPK, BCL-2 and caspase family proteins; caspase-3, caspase-7, caspase-8, and caspase-9.

Nonetheless, an anti-cancer drug called doxorubicin was observed to share mutual protein targets as sesamin. Doxorubicin is a cancer chemotherapy drug commonly used in the treatment of cancers, including leukemia. Doxorubicin can induce apoptosis in several types of cancer cells such as hepatoma cell lines (44), breast cancer cell lines (45), and acute lymphoblastic leukemia cell lines (46) via p53 and caspase activation. It also targeted programmed cell death 4 on apoptosis induction in HCT116 colon carcinoma cells and A375 melanoma cells (47). In addition, the effect of doxorubicin had been studied in various cancer cell types whose results showed the increasing level of pro-apoptotic or tumor suppressor phosphoproteins and supported its role in an anti-cancer activity (48).

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From previous study (49), it reported the effect of sesamin on the induction of both apoptosis and autophagy in MOLT-4 and NB4 leukemic cell lines. It predicted the protein-chemical interaction between sesamin and target proteins using bioinformatic analysis, which showed the connection of apoptotic protein (caspase-3) and autophagic proteins (mTOR, and ULK1). In the present study, the phosphoproteomics and mass spectrometry, followed by bioinformatic technology were used to identify novel targeted anti-cancer phosphoproteins; PARP4 and IPPK. Furthermore, apoptotic gene expression was investigated using real-time PCR. It revealed that sesamin up regulated *caspases-3, -7, -8, -9* and *BCL-2*, which were involved in both extrinsic and intrinsic apoptosis in sesamin-treated leukemic cell lines.

In conclusion, sesamin possesses an ability to enhance cell inhibition and promotes cell apoptosis in both MOLT-4 and NB4 leukemic cell lines in dose- and time-dependent manner with no significant decrease of the cell viability in normal PBMCs. The induction and execution of apoptosis process demonstrated that sesamin treatment in MOLT-4 and NB4 were involved in caspase-dependent apoptosis signaling pathway. This study reports on the effect of sesamin playing a role in apoptosis pathway through BCL-2, caspase family proteins, PARP4, and IPPK in MOLT-4 and NB4 leukemic cell. Additionally, sesamin was found to share the same target proteins with doxorubicin, which suggests potential use of sesamin in reducing the side effects or synergizing the cytotoxic effect of doxorubicin in leukemic patients. In the future, sesamin could be possibly developed as an alternative drug for leukemia treatment. However, further investigation on *in vivo* study or clinical samples is required.

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Conflicts of Interest

There are no conflicts of interest to disclose as well as no significant financial support for this research that could have influenced its outcome.

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