

# Phosphomonoester Phosphoethanolamine Induces Apoptosis in Human Chronic Myeloid Leukemia Cells

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**Abstract:** Background: Leukemia is a type of cancer that starts in the blood or blood-forming tissues. It results from the clonal proliferation of hematopoietic cells in the bone marrow and/or lymphoid tissues, which subsequently reach the peripheral circulation and can infiltrate other systems. There are many different kinds of leukemia, and treatments are different for each one. Chronic leukemia is with a slower growing than acute leukemia but could be just as life-threatening. Phospholipids are antitumor analogs, such as synthetic phosphoethanolamine, which is a phosphorylated compound capable of controlling cellular proliferation and inducing apoptosis in several types of tumor cells. Methods: K562 and K562-Lucena (MDR+) human chronic myeloid leukemia cells were treated with synthetic phosphoethanolamine (Pho-s). The viability was evaluated by sulforhodamine B (SRB) assay and cell cycle phases, apoptosis, markers expression, and mitochondrial potential were assessed by flow cytometry. Results: Tumor cells formed clusters in suspension and decreased significantly viability. The concentrations for IC<sub>50</sub>% were obtained. Pho-s treated were 43.1 mM (K562) and 145.9 mM (K562-Lucena MDR+) in a period of 24 hours. Pho-s induced changes in the distribution of cell population phases of cell cycle which showed an increase in fragmented DNA and increased markers expression involved apoptosis pathways a decrease in the G1/G0 phase. Discussion: Treatment of K562 and K562-Lucena (MDR+) chronic myeloid leukemia cells with Pho-s showed dose and time dependent cytotoxic effects. This cytotoxicity induced a decrease in proliferative capacity, mitochondrial electrical potential, and consequently release of cytochrome C; inhibition of Bcl-2 family protein expression, increase in pro-apoptotic family members Bad and Bax, dependent on p53 expression. Conclusion: This study presented a significant therapeutic potential of Phos-s in this type of leukemia through the apoptotic effects on tumor cells independently of the molecular resistance profile (MDR+).

**Key words:** Chronic myeloid leukemia cells, synthetic phosphoethanolamine, mitochondrial potential, apoptosis, cell cycle.

## 1. Introduction

Leukemic cells originate from a somatic mutation in a single stem cell or stem cells, which form the leukemic clone. The leukemic transformation can occur at different stages of lymphoid or myeloid precursor's differentiation. These aspects characterize leukemia as a disease with biological and morphological heterogeneous aspects. Leukemia can be classified as acute and chronic regarding cell maturation stage [1]. In chronic leukemias, chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL), the mutations allow the maintenance of differentiation capacity and cell maturation, with a characteristic increase in the number of mature cells in

the bone marrow and in peripheral blood. The progression is slow but followed by an accelerated phase that could develop into acute leukemia later [2, 3].

The alkyl-lysophospholipid analogs (ALPs) represent a novel class of lipids with antitumor activity. The phosphoethanolamine is a primary amine that presents high intracellular concentrations in various tissues and has been implicated in several important cellular functions, such as osmoregulation, membrane stabilization and neuromodulation [4, 5]. The phospholipids (PLs) are mostly formed by a substituted glycerol molecule, two fatty acids and a phosphate group, which makes the PLs an amphiphilic characteristic. The phosphate head is an easy reactional center, which can react with low molecular weight structures (e.g., serine, ethanolamine, glycerol, choline, etc.) [6]. Phospholipids derived from ethanolamine are

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essential structural components of cell membranes and play regulatory roles in cell division, signaling, activation, autophagy, and phagocytosis [7].

Synthetic phosphoethanolamine (Pho-s) is a phosphoric ester known as aminoethyl ester phosphoric that previously was synthesized by our group. Recent studies showed that treatment with Pho-s presented an antitumor effect on murine melanoma B16F10 cells [8], human breast adenocarcinoma MCF7 [9], leukemic cells [10], without causing any apparent effect on normal cells [11, 12].

The aim of this study is to verify the cell cycle modifications that are predominantly involved in apoptosis and regulation of cell cycle progression in two leukemic cell lines: K562 and K562 Lucena MDR+ treated with phosphomonoester phosphoethanolamine in order to identify and characterize the molecules involved in the regulation of cell death in myeloid human leukemia, molecules related to apoptosis, such as activation of caspases, molecules involved in the electrical activity of the mitochondria, and the release of pro-apoptotic factors such as Bax and cytochrome C.

## **2. Material and Methods**

### *2.1 Cell Lines*

Synthetic phosphoethanolamine was synthesized and supplied by the Laboratory of Chemistry and Polymers Technology, University of São Paulo, São Carlos, Brazil. K562 human tumor cells were purchased from the American Type Culture Collection, ATCC® CCL-243™ (ATCC, Baltimore, MD, USA). K562-Lucena tumor cell MDR+ awarded by Vivian Rumjanek, MD (Institute of Medical Biochemistry, Federal University of Rio de Janeiro) maintained and stored in the bank cell laboratory.

### *2.2 Culture*

K562 and K562-Lucena (MDR+) human chronic myeloid leukemia cells maintained in RPMI-1640 medium, with 10% fetal bovine serum, 100 units/mL

penicillin and 100 mg/mL streptomycin. Cells were cultured at 37 °C with 5% CO<sub>2</sub>. The K562 tumor cells are derived from CML patient cells that carry BCR-ABL mutation.

### *2.3 Determination of Cytotoxic Activity by Sulforhodamine B (SRB)*

K562 and K562 Lucena MDR+human tumor cells were seeded in 96-well tissue culture plates at 10<sup>4</sup> cells per well and treated with different concentrations of Pho-s diluted in saline solution. After 24 h of treatment, they are fixed with 50 µL of ice-cold trichloroacetic acid (TCA) and maintained at 4 °C for 1 h. The cells were washed five times with distilled water to remove the supernatant and centrifuged at 1,500 rpm for 5 min. After drying, 100 µL of 0.2% SRB solution in 1% acetic acid was added to each well maintaining the plate for 10 min at room temperature. Then, it was added 100 µL buffer (10 mMTris base, pH = 10.5) to each well for further reading of the absorbance at the wavelength of 550 nm.

### *2.4 Determination of Cellular Viability*

The cells were treated with Pho-s in the 12 and 24 h and then removed from the plates for cell counting. The tubes containing suspension cells were centrifuged at 1,500 rpm for 5 min. The supernatant was discarded and the cells were resuspended in 1 mL of culture medium. Then, automated cellular viability analyses were performed (Vi-Cell™MXR-Analyzer; Beckman Coulter Inc., Fullerton, California, EUA). The apparatus performed an automated exclusion assay using the 0.2% trypan blue method (cells with the compromised plasma membrane allow trypan blue to enter and are stained blue by the analyzer), providing the percentage of cell viability.

### *2.5 Cell Cycle Phases Analysis*

K562 and K562-Lucena (MDR+) cells were treated with Pho-s for 12 and 24 h. Cultured cells were then collected and fixed with cold 70% ethanol/20 mg/mL

RNase (Sigma) and stored at  $-20^{\circ}\text{C}$  overnight. Cells were incubated at  $37^{\circ}\text{C}$  for 45 min in 0.5 mL PBS and then stained with 1 mg/mL propidium iodide (PI) and 100 mg/mL, and Rnase for 30 min at  $37^{\circ}\text{C}$ . Quantification of DNA content (10,000 events) was performed for each sample by flow cytometry. Data were acquired using CellQuest software (Becton Dickinson) and analyzed using MoDFIT Software.

### *2.6 Measurement of Mitochondrial Membrane Potential*

The mitochondrial membrane potential was measured by rhodamine123 prime monitored by flow cytometry. K562 and K562-Lucena (MDR+) human tumor cells at the density of  $10^5$  cells were plated in six well plates and treated with Pho-s for 24 h. Rhodamine 123 was added at 100 mg/L 30 min before end of treatment. After washing with PBS, the cells were analyzed using an FACScan flow cytometry system (Scalibur-Becton Dickinson, San Jose, CA). A total of 10,000 cells/sample were analyzed and the mean fluorescence intensity (FL1-H channel) and percentage of cells in histogram distribute of each population were recorded.

### *2.7 Apoptosis and Necrosis Analyses (Annexin V/PI) by Flow Cytometry*

The cells were treated with Pho-s for 24 h and then washed and PBS and resuspended in 100  $\mu\text{L}$  with PBS. K562 and K562-Lucena human tumor cells were incubated for 30 min with 1  $\mu\text{g}$  of Annexin V-FITC diluted in binding buffer and 18  $\mu\text{g/mL}$  propidium iodide (PI) solutions. The reading of the amount of Annexin V expression (apoptosis) and PI (necrosis) will be held in flow cytometer—FACScalibur (BD) in fluorescence intensity FL1-H/FL2-H. The results will be analyzed using WinMDI 2.8 Software.

### *2.8 Evaluation of Markers by Flow Cytometry*

K562 and K562-Lucena (MDR+) human tumor cells

treated with Pho-s and control groups were incubated for 1 h at  $4^{\circ}\text{C}$  with 1  $\mu\text{g}$  of specific antibody. Markers involved in cell death and regulators of cell cycle progression were used (Caspase 3 and 8 active, Bax, Bad, Bcl-2, p53, p21, p27, cytochrome C, Cyclin D1). After this period the cells were centrifuged at 1,500 rpm and washed with ice-cold PBS and 0.2% BSA for 2 times. The supernatant was discarded and the pellet resuspended in 200  $\mu\text{L}$  of Fac's buffer containing 0.1% paraformaldehyde. The reading and analysis of the expression of receptors on the cell surface of tumor cells were performed in an FACScalibur flow cytometer (BD) in FL-1 and the fluorescence intensity histograms were acquired and analyzed using Cell-Quest-BD.

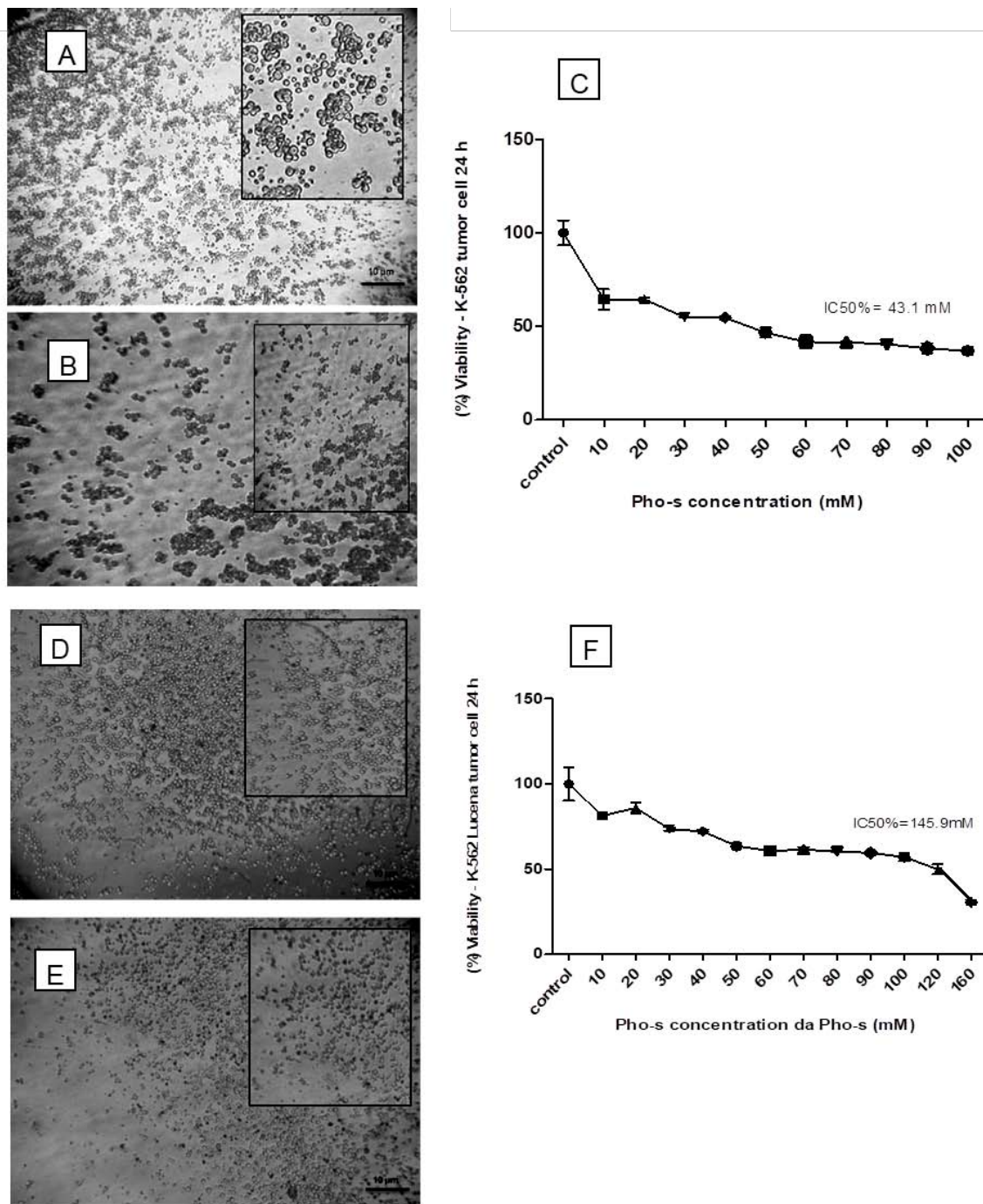
## **3. Results**

### *3.1 Determination of Cytotoxic Activity by Sulforhodamine B (SRB)*

After 24 h of treatment, human leukemia cells were incubated with Pho-s at concentrations of 10 to 160 mM, in the time of 24 h. K-562 cells lysis were morphologically observed with cell debris formation at 40 mM of Pho-s concentration. The concentration of Pho-s to obtain IC<sub>50</sub>% was 43.1 mM. K-562-Lucena (MDR+) cells lysis were morphologically observed cell debris formation and cytotoxicity at a concentration of 120 mM. The concentration of Pho-s to obtain IC<sub>50</sub>% was 145.9 mM (Fig. 1).

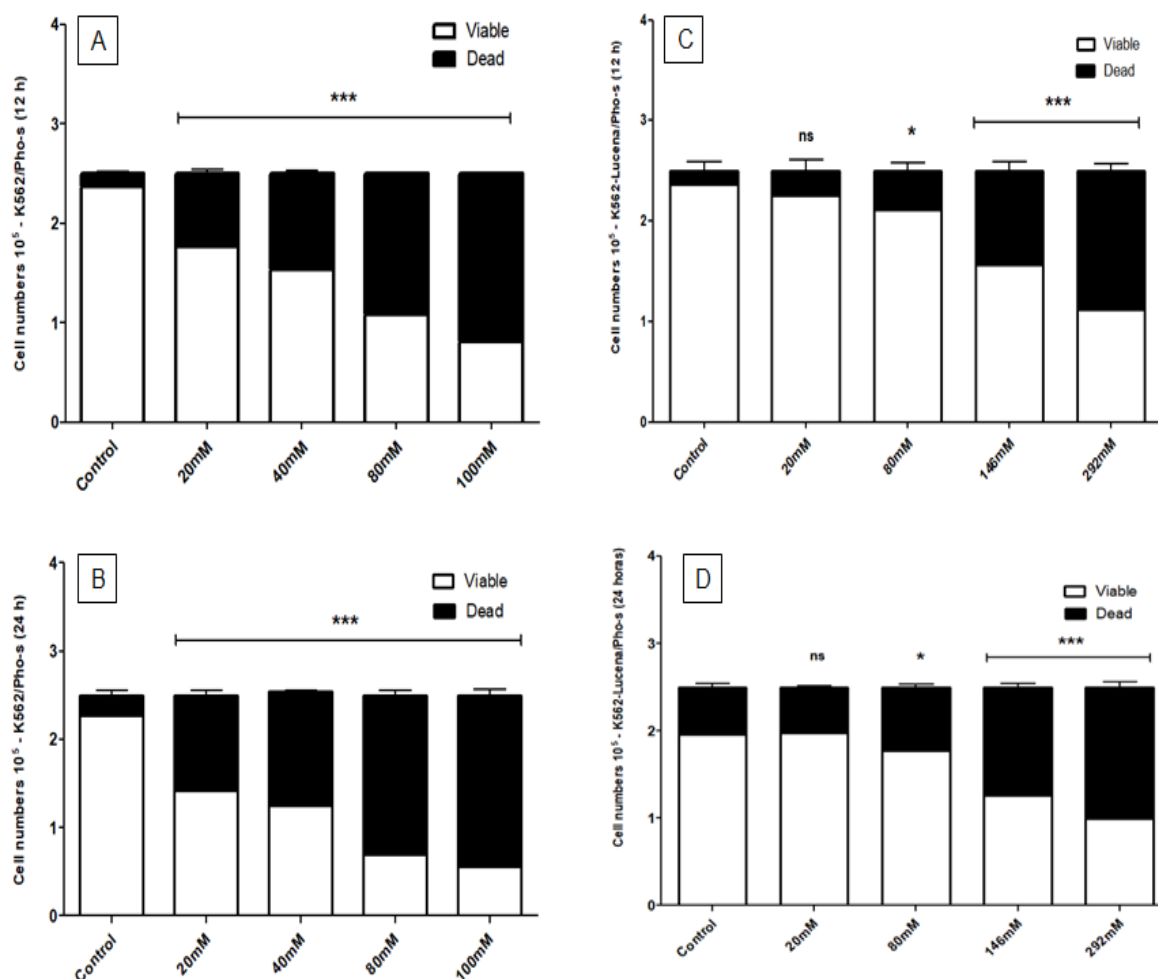
### *3.2 Evaluation of Cell Viability*

Cell viability was determined to be excluded by trypan blue 0.2% by the Vi-Cell™ XR-Analyzer system (Beckman Coulter Inc., Fullerton, California, USA). The concentration used in all experimental conditions was obtained from IC<sub>50</sub>% inhibitory concentration; however, the cellular viability of the treated groups compared to the untreated control group at various concentrations and at different treatment time periods significantly decreased (Fig. 2).



**Fig. 1** Cellular morphologically aspects of K562 and K562-Lucena (MDR+) human chronic myeloid leukemia cells treated with Pho-s. (A) control and (B) treated groups with Pho-s after 24 h, obtained in an inverted microscope light (magnitude 40×). After treatment, cells showed more rounded shape, the formation of apoptotic body, and cells' fragmentation. (C) Scatter plots of the mean values  $\pm$  SD of cell viability on a logarithmic scale of the tumor cells after the 24 h treatment with Pho-s to obtained IC50% value using GraphPad Prism 5 Software of three independent experiments. (D) control and (E) treated groups with Pho-s after 24 h, obtained in an inverted microscope light (magnitude 40×). After treatment, there is a significant decrease in cell density and their morphological appearance showed more rounded cells' shape, the formation of apoptotic body, and cells fragmentation. (F) Scatter plots of the mean values  $\pm$  SD of cell viability on a logarithmic scale of the tumor cells after the 24 h treatment with Pho-s to obtain the IC50% value using GraphPad Prism 5 Software. Mean  $\pm$  SD of three independent experiments.

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**Fig. 2** Viability of K562 and K562 Lucena MDR+ human leukemic cell lines after treatment with Pho-s at various concentrations and time points. (A) K562 tumor cells treated with Pho-s after 12 h, (B) 24 h. K562-Lucena (MDR+) tumor cells treated with Pho-s after (A) 12 h (B) 24 h (GraphPad Prism 5 Software). Mean  $\pm$  SD of three independent experiments.

### 3.3 Analysis of the Cell Cycle Phases by Flow Cytometry

The present study analyzed the cell cycle phase distribution to understand the mechanisms of Pho-s induced K562 and K562 Lucena MDR+ to cell apoptosis. K562 cells were treated with Pho-s for 12 and 24 h, and the cell cycle was analyzed using flow cytometry according to the DNA content. Pho-s was tested to evaluate their ability to modify the distribution profile of the cell populations in the cell cycle phases. These concentrations were chosen from the calculation of IC<sub>50</sub> obtained at different times. The percentage of K562 and K562-Lucena (MDR+) tumor cells in

different phases of the cell cycle was quantified after treatments. Treatments after 12 and 24 h with Pho-s presented significant changes in cell cycle phases compared to the control group. The G<sub>0</sub>/G<sub>1</sub> phase decreased significantly, while fragmented DNA increased (Figs. 3 and 4).

### 3.4 Measurement of Mitochondrial Membrane Potential

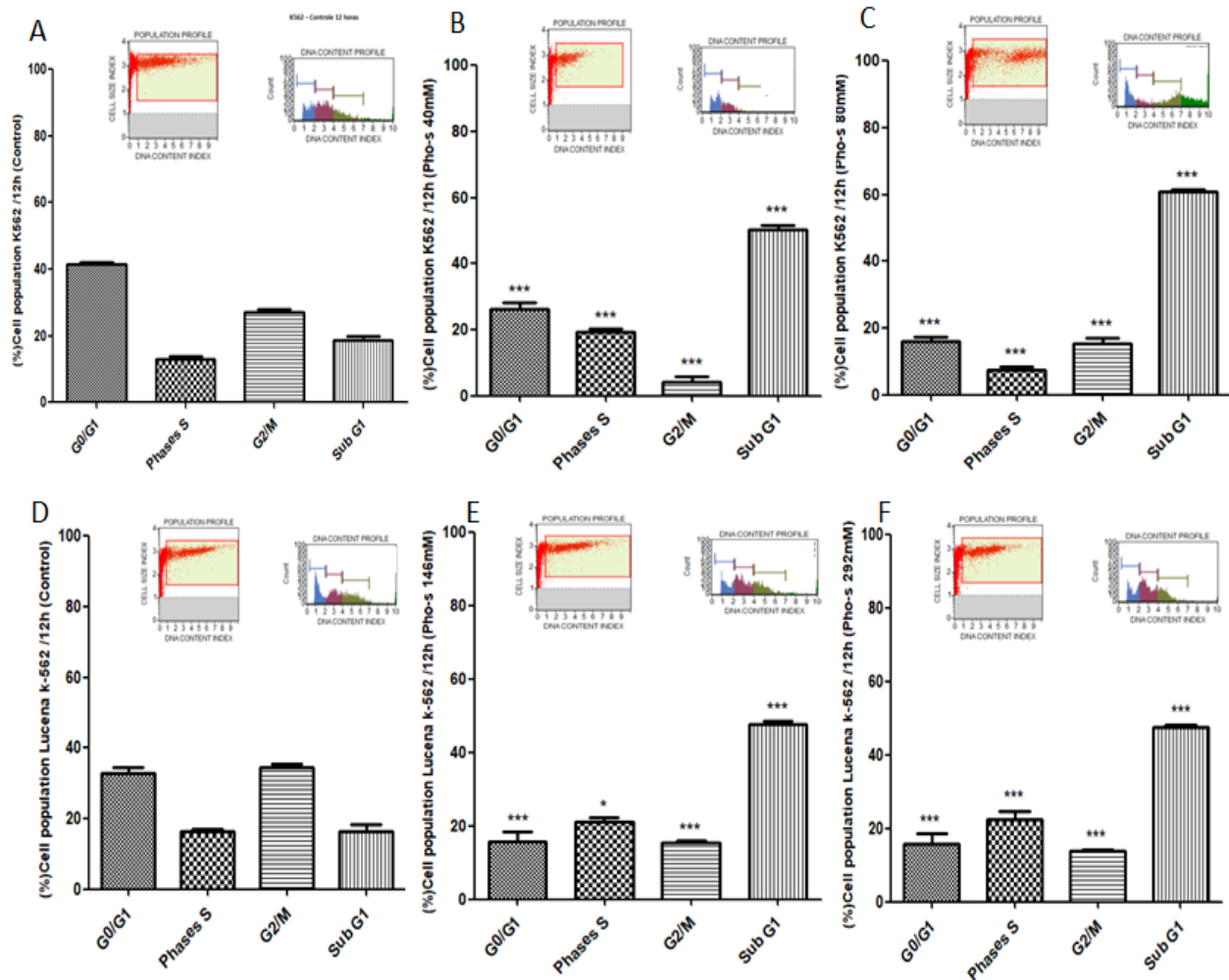
The analyses on a flow cytometer after labeling by fluorescent probe rhodamine 123 demonstrated that treatment with Pho-s significantly decreased mitochondrial electrical potential in K562 and K562-Lucena (MDR+) human tumor cells when

compared to the control group, especially at a concentration of 40 and 120 mM Pho-s, respectively (Fig. 5).

### 3.5 Evaluation of Apoptotic Activity with Annexin V/PI

To further determine whether Pho-s treated K562 and K562 Lucena MDR+ cells underwent apoptosis,

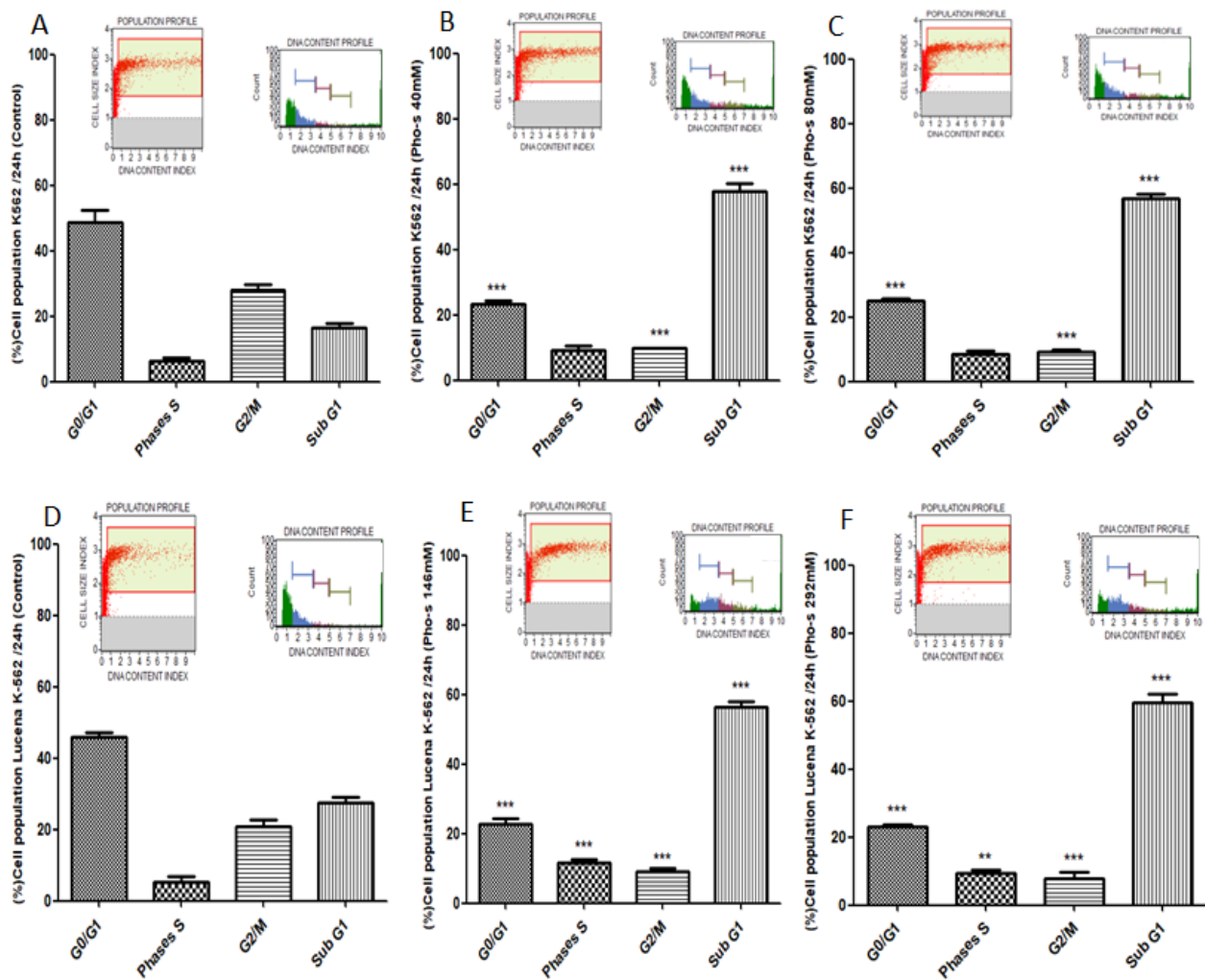
the cells were stained using double-staining with Annexin V and PI. K562 and K562-Lucena (MDR+) human tumor cells were treated with Pho-s for 24 h at concentrations of 40 and 80 mM or 146-292 mM, respectively. Pho-s induces a significant increase in the percentage of dead cells in late, besides early apoptosis had been observed in a dose-dependent manner (Fig. 6).



**Fig. 3** K-562 human chronic myeloid leukemia cells representative histograms and graphs of cell cycle phases distributions (means  $\pm$  SD). (A) control and treated groups (B) Pho-s 40 mM and (C) Pho-s 80 mM. K-562-Lucena (MDR+) human leukemia cell. (D) control and treated groups (E) Pho-s 146 mM and (F) Pho-s 292 mM. Statistical significance \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ , obtained by the Kruskal-Wallis non-parametric test, followed by Dunn's multiple comparison tests. Mean  $\pm$  SD of three independent experiments.



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**Fig. 4** K-562 human leukemia cell representative histograms and graphs of cell cycle phases (means  $\pm$  SD). (A) control and 24 h treated groups (B) Pho-s 40 mM and (C) Pho-s 80 mM. K-562-Lucena (MDR+) human leukemia cell (D) control and 24 h treated groups (E) Pho-s 146 mM and (F) Pho-s 292 mM. Statistical significance \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ , obtained by the Kruskal-Wallis non-parametric test, followed by Dunn's multiple comparison tests. Mean  $\pm$  SD of three independent experiments.

## 3.6 Evaluation of Markers by Flow Cytometry

K562 and K562-Lucena (MDR+) human tumor cells were analyzed cell cycle progression markers (Caspase 3 and 8 active, Bax, Bad, Bcl-2, p53, p21, p27, cytochrome C, Cyclin D1). Data were acquired in the flow cytometer (FACSCalibur) and analyzed by WinMDI version 2.9 software. Bcl-2 decreased

significantly in the group treated with Pho-s when compared to the control group (Figs. 7-9). However, Bax and Bad increased significantly. Caspases 3 and 8 activities were increased in the group treated with Pho-s. Markers cyclin D1, p53, p21, p27, and cytochrome C had a significant increase in the group treated with Pho-s when compared to the control group (Figs. 10-12).

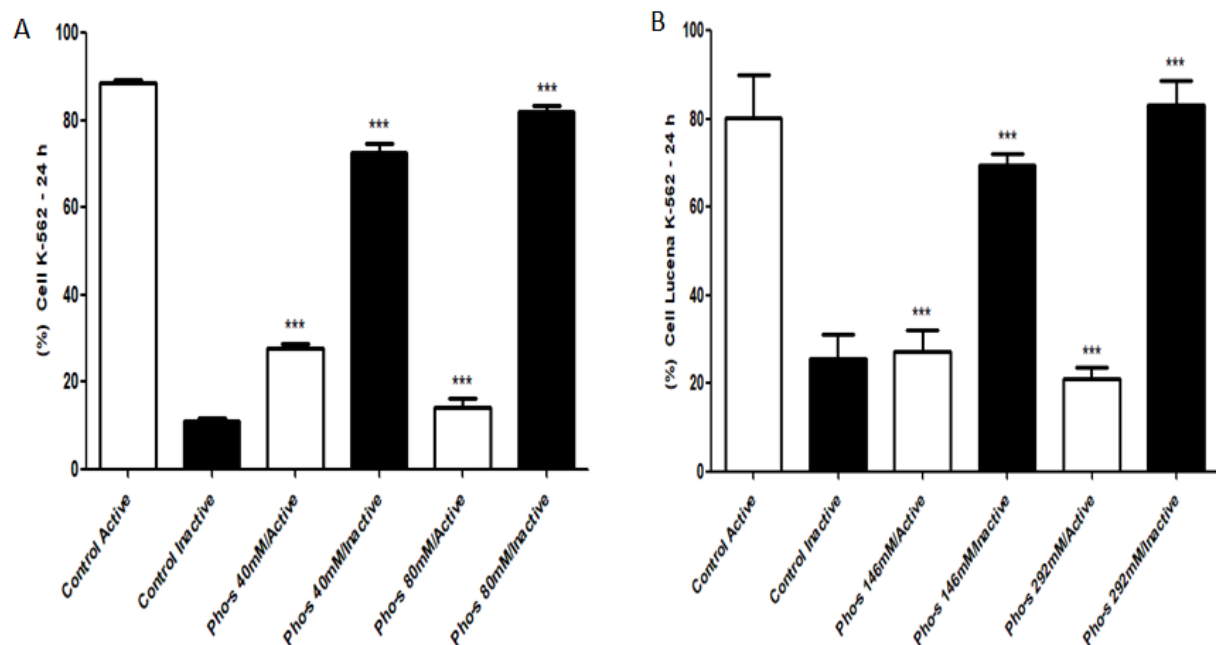


Fig. 5 The average values  $\pm$  SD of the percentage of the mitochondrial electric potential of human leukemia cell. (A) After 24 h treated Phos-s on K562 and (B) K562-Lucena (MDR+) human leukemic cells. Significance values  $p < 0.05$  and  $p *** < 0.01$ , ANOVA obtained by variation of the test followed using multiple test-Turkey Kremer. Mean  $\pm$  SD of three independent experiments.

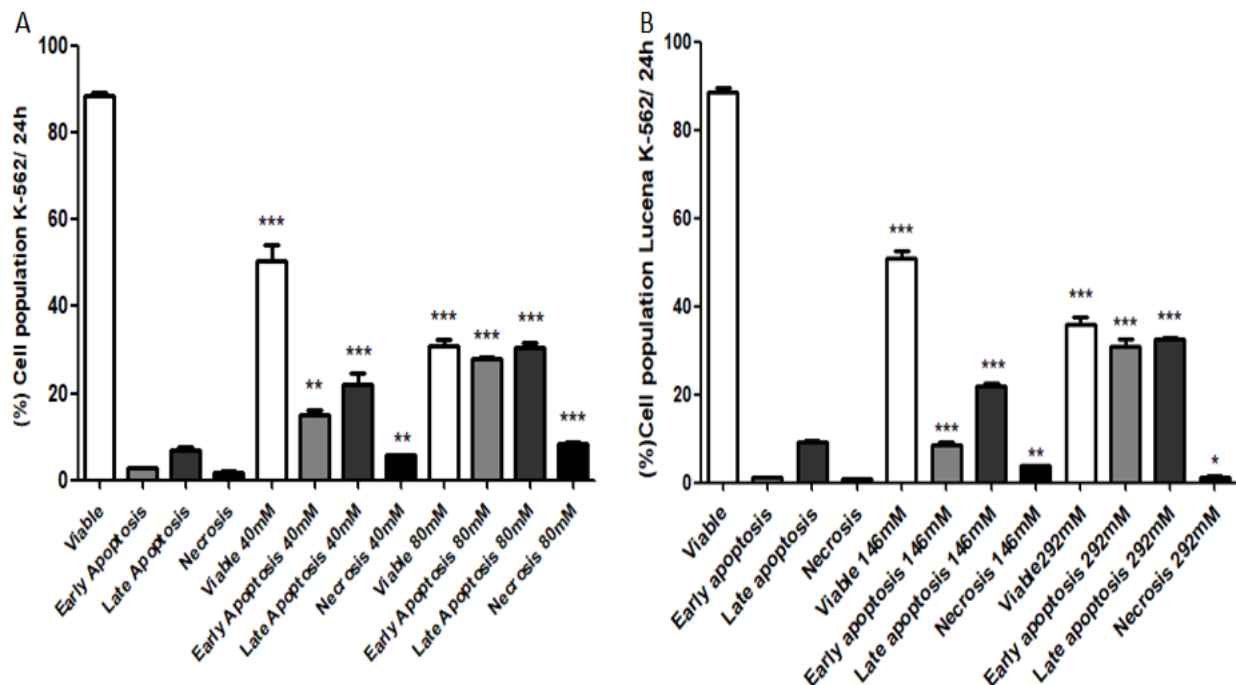


Fig. 6 Cytometric analyses of the determination of the initial and late apoptosis or necrosis of the K-562 human leukemic cells. (A) and K-562-Lucena (MDR+) leukemic cells (B) after 24 h. Statistical significance  $* p < 0.05$ ,  $** p < 0.01$  and  $*** p < 0.001$ , obtained by the Kruskal-Wallis non-parametric test, followed by Dunn's multiple comparison tests. Mean  $\pm$  SD of three independent experiments.



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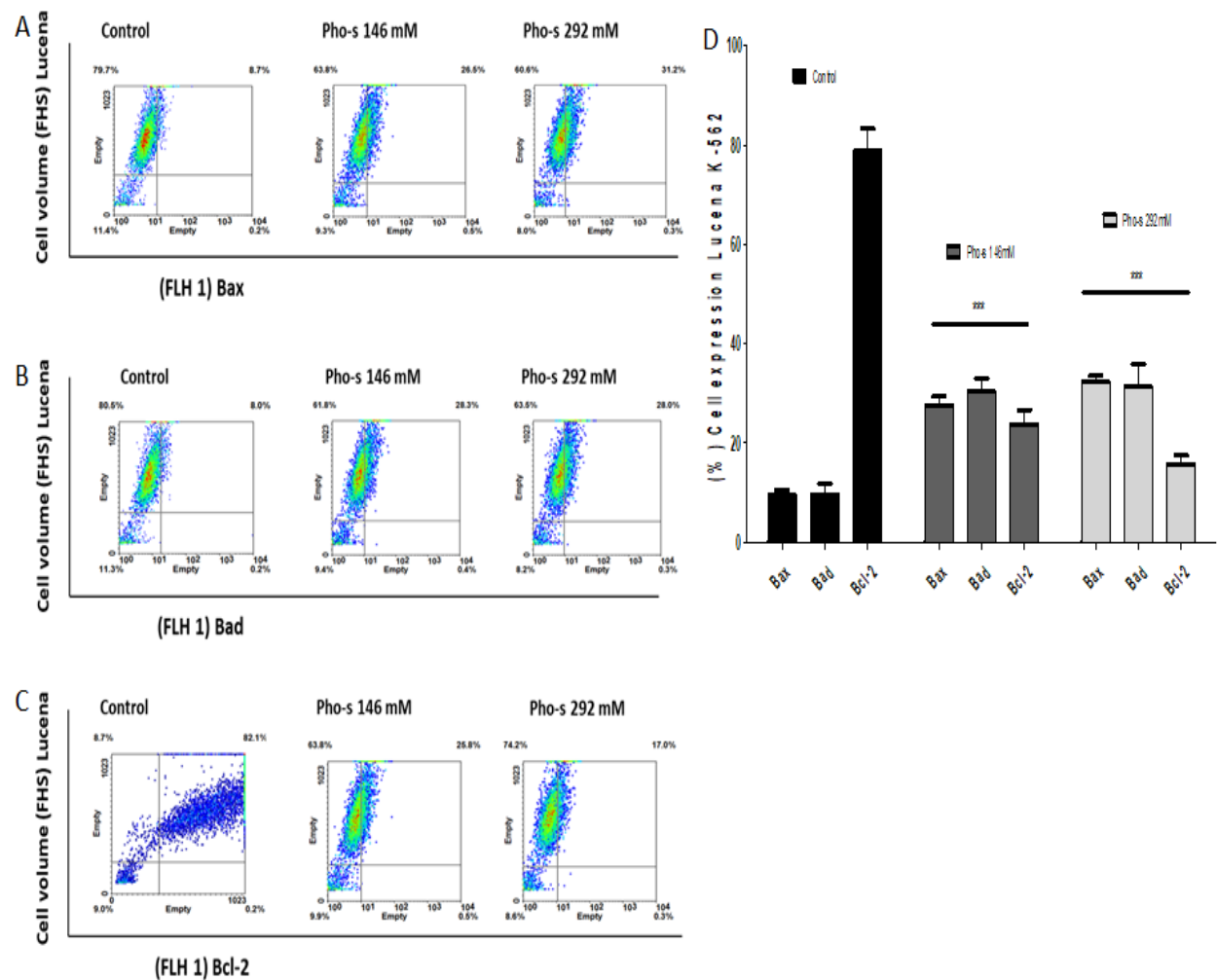
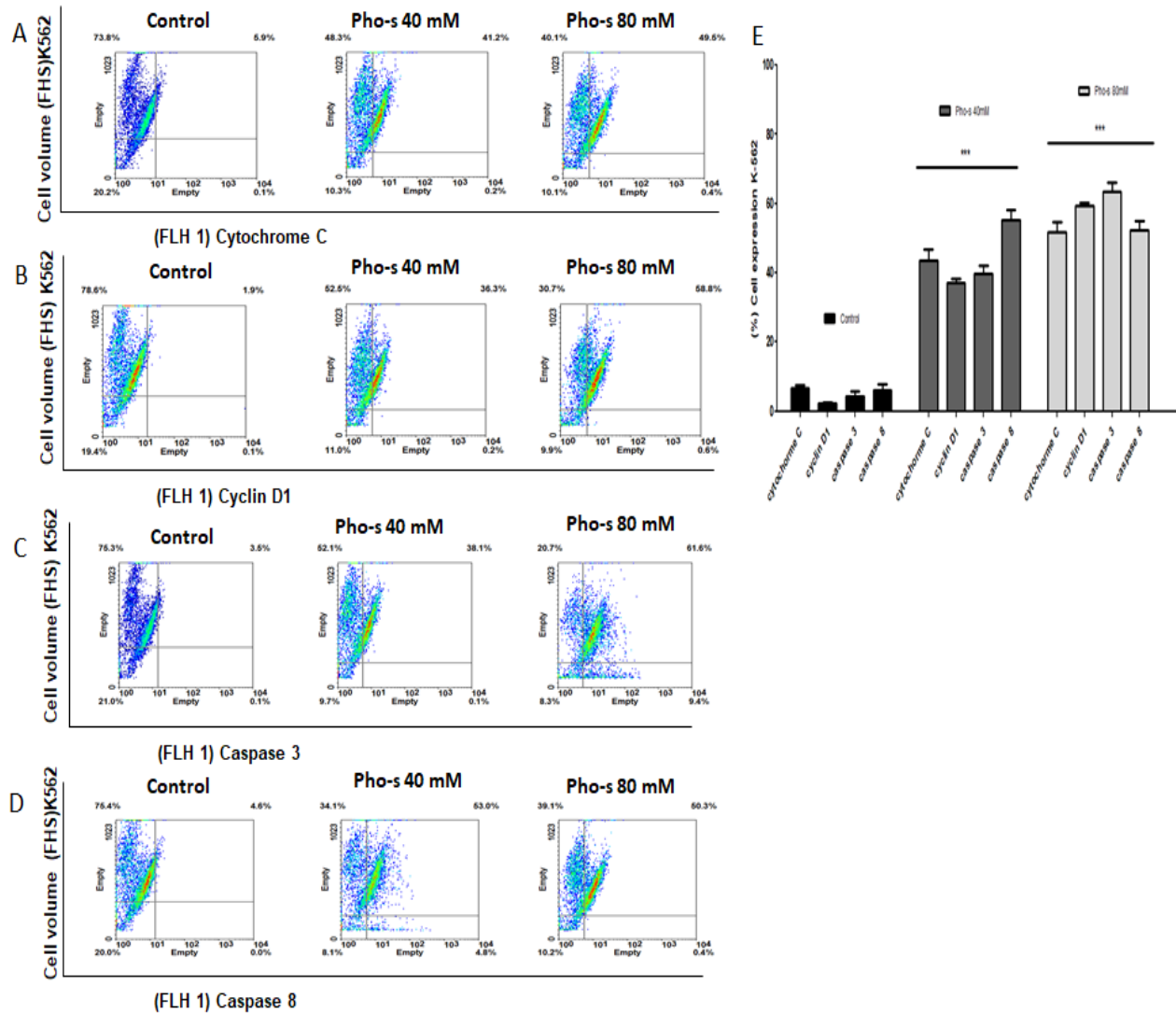
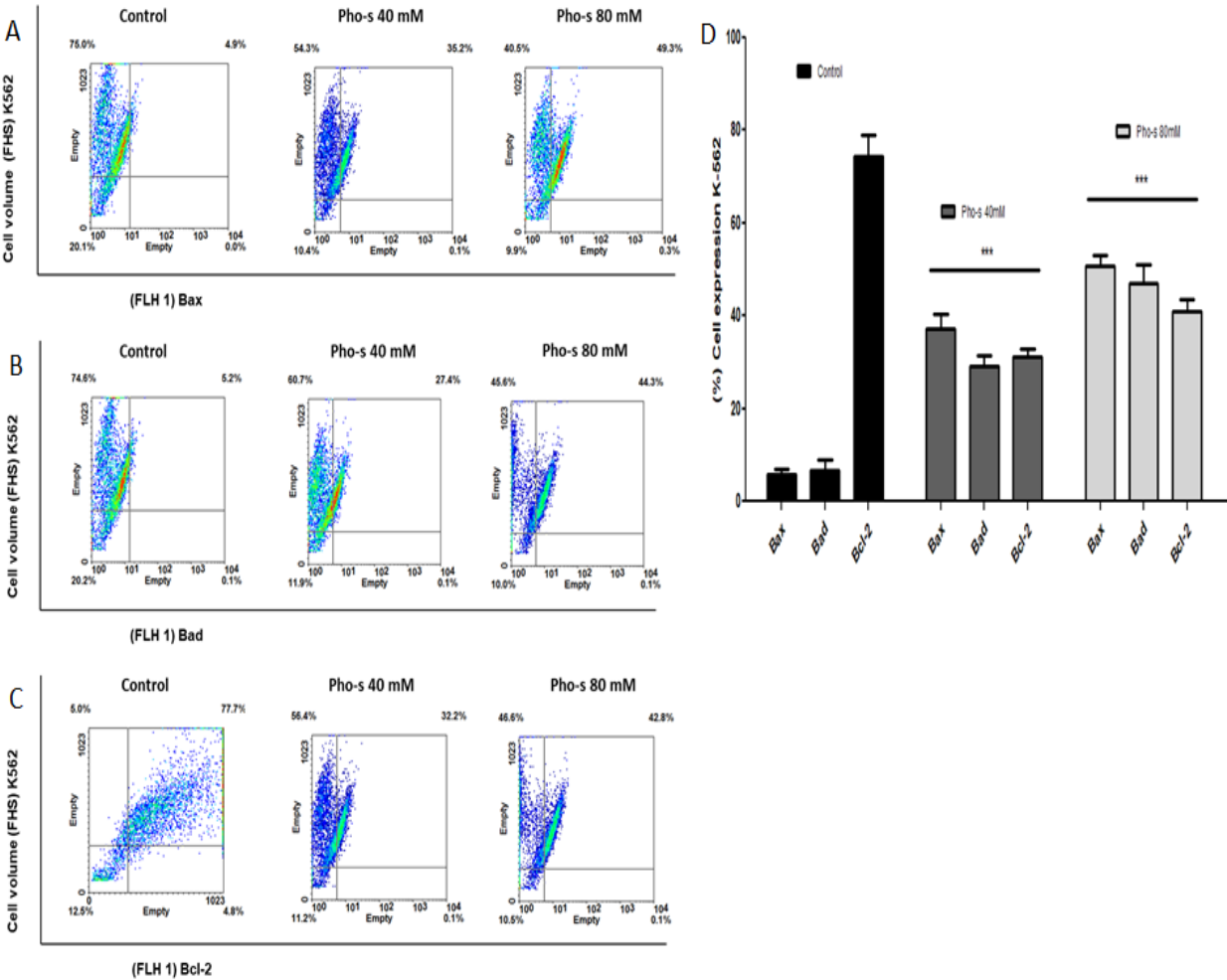


Fig. 7 Markers expressions histograms involved in apoptosis in K562 human leukemic cells. (A) Bax; (B) Bad, and (C) Bcl-2 in K562 human leukemic cells obtained by flow cytometry; (D) Significance values;  $p^* < 0.05$  and  $p^{***} < 0.01$ , ANOVA obtained by variation of the test followed using multiple test-Turkey Kremer. Mean  $\pm$  SD of three independent experiments.

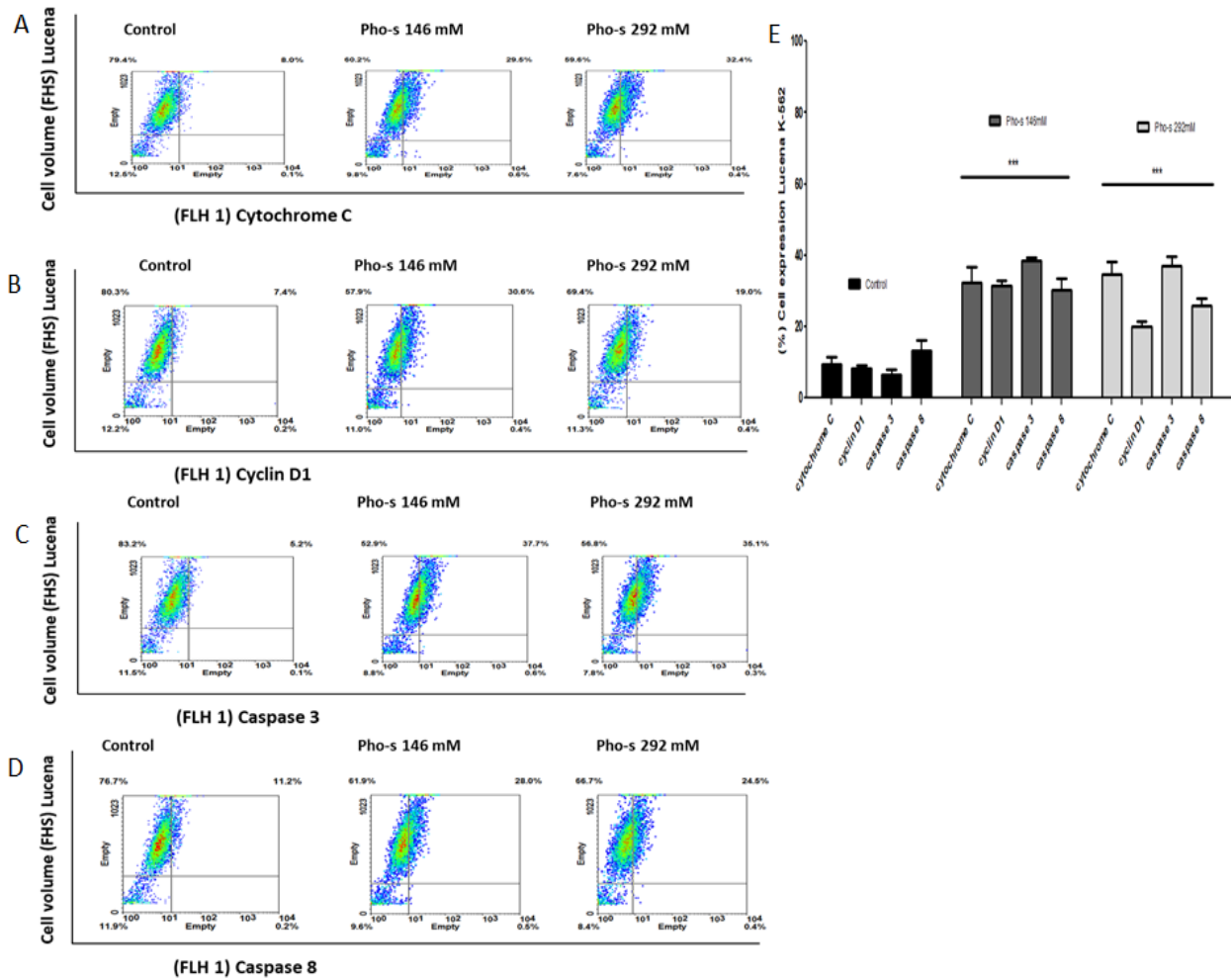


**Fig. 8** Markers expressions histograms involved in apoptosis in K562-Lucena (MDR+) human leukemic cells. (A) Bax; (B) Bad and (C) Bcl-2 in K562-Lucena (MDR+) human leukemic cells obtained by flow cytometry. (D) Significance values  $p^* < 0.05$  and  $p^{***} < 0.01$ , ANOVA obtained by variation of the test followed using multiple test-Turkey Kremer. Mean  $\pm$  SD of three independent experiments.

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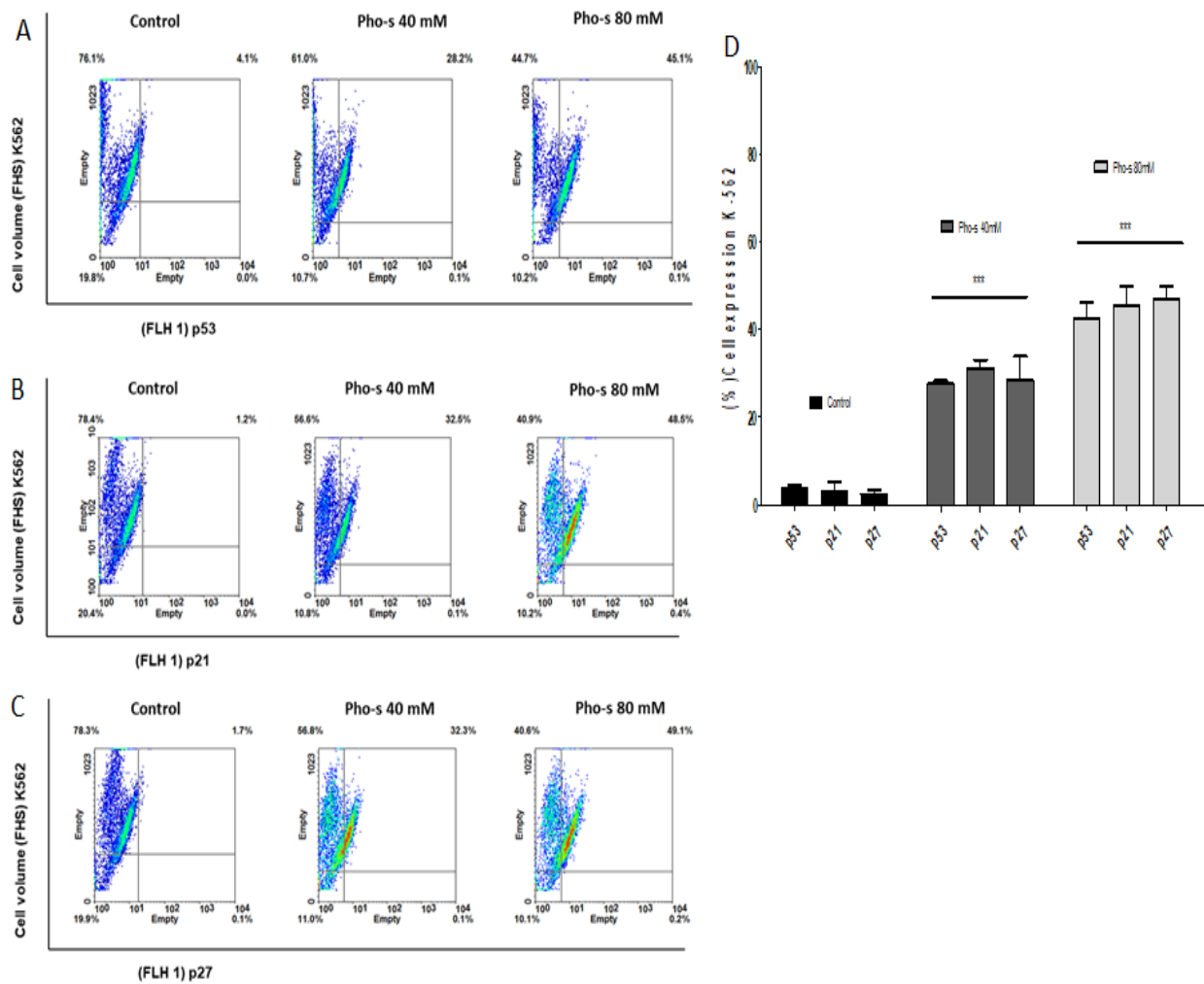


**Fig. 9** Markers expressions histograms involved in apoptosis and progression cellular proliferation in K562 human leukemic cells. (A) cytochrome C, (B) cyclin D1, (C) caspase 3 and (D) caspase 8 in K562 human leukemic cells obtained by flow cytometry. (E) Significance values  $p^* < 0.05$  and  $p^{***} < 0.01$ , ANOVA obtained by variation of the test followed using multiple test-Turkey Kremer. Mean  $\pm$  SD of three independent experiments.

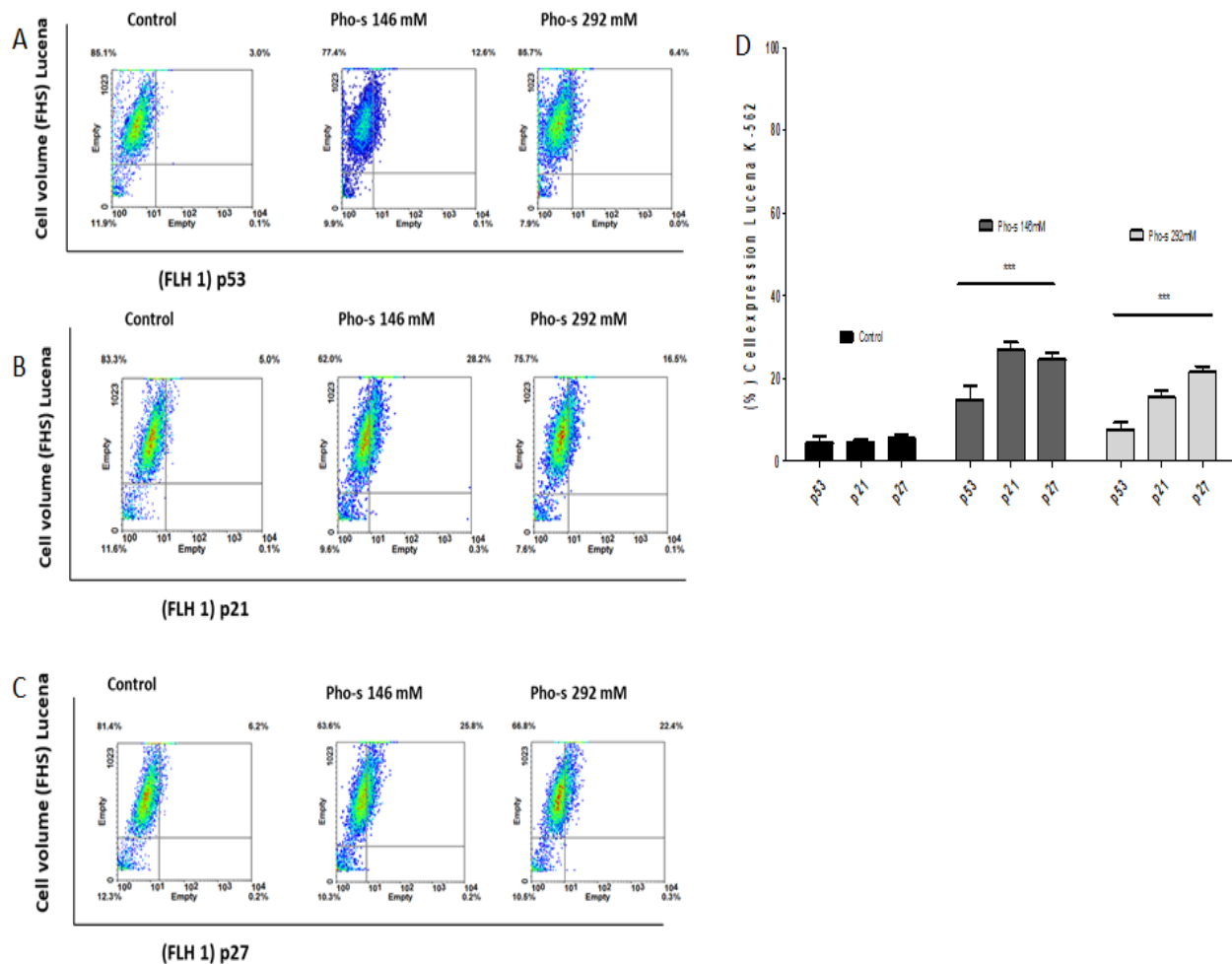


**Fig. 10** Markers expressions histograms involved in apoptosis and progression cellular proliferation in K562-Lucena (MDR+) human leukemic cells. (A) cytochrome c, (B) cyclin D1, (C) caspase 3 and (D) caspase 8 in K562-Lucena (MDR+) human leukemic cells obtained by flow cytometry. (E) Significance values  $p < 0.05$  and  $p < 0.01$ , ANOVA obtained by variation of the test followed using multiple test-Turkey Kremer. Mean  $\pm$  SD of three independent experiments.

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**Fig. 11** Markers expressions histograms involved in apoptosis and control proliferation in K562 human leukemic cells. (A) p53, (B) p21 and (C) p27 in K562 human leukemic cells obtained by flow cytometry. (D) Significance values  $p^* < 0.05$  and  $p^{***} < 0.01$ , ANOVA obtained by variation of the test followed using multiple test-Turkey Kremer. Mean  $\pm$  SD of three independent experiments.



**Fig. 12** Markers expressions histograms involved in apoptosis and control proliferation in K562-Lucena (MDR+) human leukemic cells. (A) p53, (B) p21 and (C) p27 in K562-Lucena (MDR+) human leukemic cells obtained by flow cytometry. (D) Significance values  $p^* < 0.05$  and  $p^{***} < 0.01$ , ANOVA obtained by variation of the test followed using multiple test-Turkey Kremer. Mean  $\pm$  SD of three independent experiments.

## 4. Discussion

This study evaluated the potential cytotoxicity of synthetic phosphoethanolamine (Pho-s) in human chronic myeloid leukemia tumor cells. Several studies have been published showing that antineoplastic phospholipids act on tumor cell membranes, interfering with the turnover of phospholipids. Esther linkages, which are not metabolized, can interfere with signaling lipids, causing apoptosis in malignant tumor cells due to its stability [13].

Corroborating data, Pho-s was cytotoxic to all tumor cell lines studied by our research group: EAT (ehrlich

ascites tumor); B16F10 cells (murine melanoma); MCF7 cells (human breast cancer); H292 cells (lung cancer); SKMEL-28 and MEWO cells (human melanoma). Besides that, treatment with Pho-s was not cytotoxic to normal cells such as fibroblasts and endothelial cells (MENEGUELO, 2007 [14, 15]). The results of cytotoxicity tests indicate that Pho-s promotes anti-tumor effects through a mechanism that appears to be common to all strains without promoting significant cytotoxic effects on normal cells.

In this study, cytotoxic effects and mechanisms of apoptosis induction were evaluated after treatment with Pho-s. Pho-s promoted antitumor effects with IC 50%



values for 43.1 mM K-562 and 145.9 mM K-562 Lucena (MDR+) tumor cells. The sensitivity level for the two strains was different, and probably depended on the resistance of the mutagen profile, in particular, MDR+ and aggressiveness. Furthermore, the activity of BCR-ABL has several effects on leukemia' cells, causing an increase in proliferation and a reduction in apoptosis, leading to the malignant growth of groups of hematopoietic stem cells.

In phase G1 of the cycle, the cell undergoes internal and external monitoring to ensure proper conditions for the division. To ensure that such intra and extracellular conditions are adequate for the correct duplication of the genetic material in the S phase, there are two checkpoints in G1, called the restriction point and control point [16]. The analysis of cell cycle phases by flow cytometry showed that leukemic tumor cells treated with different concentrations of Pho-s decreased the proportion of cells in the G1/G0 phase and increased fragmented DNA, independent phenotype MDR.

K-562 and K-562-Lucena (MDR+) human leukemic cells treated with Pho-s showed an increase in the proportion of cells in the late and early apoptosis process, and a significant increase in the percentage of necrotic cells. These data support the hypothesis that Pho-s does not induce damage to the cell membrane and, rather, it binds to cell membrane receptors, lipids rafts, which are phospholipid ligands and apoptosis inducers, independent of the phenotype of resistance to multiple drugs.

The most studied mechanism of programmed cell death is apoptosis, which occurs through the intrinsic and extrinsic pathways. The intrinsic pathway, or mitochondrial, is activated by signals of intracellular stress that damage the DNA. This stimulus increases the permeability of the mitochondrial membrane by modifying the interaction between Bcl-2 family proteins that interact with the mitochondrial membrane-dependent ion channels. Bcl-2 proteins have pro-apoptotic (Bak, Bax or Bok) or anti-apoptotic

functions (Bcl-2, Bcl-XL or Mcl-1). Apoptosis can be initiated by activation of a death receptor on the plasma membrane by the effects of Bcl-2 family proteins on mitochondria, or by direct toxic effects on mitochondria leading to loss of mitochondrial membrane potential and release of Pro-apoptotic mitochondrial proteins. Activation of different caspases may initiate or amplify the apoptotic response depending on mitochondria, and independent caspase pathways [17].

Pho-s positively modulated the expression of Bcl-2, Bad and Bax, associated with decreased mitochondrial potential activities and the release of cytochrome C. Pho-s is a phosphomonoester with pro-apoptotic properties in leukemic cells K562 and K562-Lucena (MDR+). Data supporting the findings in this work, the Pho-s modulated the activities of mitochondrial electrical potential, cytochrome c release and Bad and Bax increase, which confirm the proportion of cells in apoptosis, positively labeled with Annexin-V. ALPs may induce apoptosis in S49 lymphoma cells related to the ability to inhibit the CTP/phosphocholine enzyme involved in the synthesis of phosphatidylcholine [18].

These data corroborate with the findings by our research group previously, in which Pho-s induces *in vitro* apoptosis on leukemic cell lines through a mitochondrial pathway [10].

P53 tumor suppressor protein is a key protein in apoptosis and plays an important role in signal transduction pathways in several cell types, including fibroblasts and vascular endothelial cells. The role of p53 is of great importance in genotoxic stress where it modulates and integrates several types of response that control apoptosis, cell cycle arrest, senescence, and other physiological processes. The transcriptional activity of p21 is under the control of p53 protein. P21 will also compete with cyclin D with the same intention to cause cell cycle arrest. P27 responds to growth suppressors and will compete with the cyclin E/CDK-2 complex, also causing cell cycle arrest at the G1/S restriction point, modifying the proportion of

quiescent/senescent cells [19]. The markers studied in this work involved in the control of cell cycle progression, p53, p21, and p27 showed a modulating effect on groups of cells treated with Pho-s.

Treatment of K562 and K562-Lucena (MDR+) chronic myeloid leukemia cells with Pho-s showed dose-dependent and time-dependent cytotoxic effects. This cytotoxicity induced a decrease in proliferative capacity, mitochondrial electrical potential, and consequently release of cytochrome C; inhibition of Bcl-2 family protein expression, increase in pro-apoptotic family members Bad and Bax, dependent on p53.

## 5. Conclusions

This study presented a significant therapeutic potential of Phos-s in this type of leukemia through the apoptotic effects of Pho-s on tumor cells independently of the molecular resistance profile (MDR+).

The use of combinations of antitumor drugs such as Pho-s is promising in this experimental leukemia cell model. We expect to evaluate in the future the association of Pho-s with existing chemotherapy regimens and protocols, which could increase overall survival rates or minimize side effects, especially neutropenias, in the treatment of leukemia and lymphoma. Synthetic phospholipids, such as Pho-s are particularly interesting since they are regulators of signaling, growth and differentiation pathways, which alone or in combination can amplify conventional therapeutic responses. The ability to modulate pro-apoptotic proteins may enhance treatments without toxicity associated with conventional cytotoxic agents, which could have a useful clinical approach, in particular in hematological tumors.

## Acknowledgments

Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq (Process number 306124/2015-7); Fundação de Amparo à Pesquisa do Estado de São Paulo—FAPESP (Process number 2016/15596-4).

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

TOC had substantial experimental contributions to conception and study design, acquisition of data, analysis, and interpretation of data; and participated in drafting paper. MGLS had substantial experimental contributions, acquisition, analysis, and interpretation of data; and participated in drafting paper. GOC was responsible to synthesize the compound used in the paper. DAM was responsible to research, and DM had substantial experimental contributions since conception, acquisition, analysis, and interpretation of data to drafting the paper.

All authors read and approved the final manuscript.

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