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MAHBUB, Amani A, LE MAITRE, Christine <http://orcid.org/0000-0003-4489-7107>, HAYWOOD-SMALL, Sarah <http://orcid.org/0000-0002-8374-9783>, MCDOUGALL, Gordon J, CROSS, Neil <http://orcid.org/0000-0003-2055-5815> and JORDAN-MAHY, N.

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Differential Effects of Polyphenols on Proliferation and Apoptosis in Human Myeloid and Lymphoid Leukemia Cell Lines

Amani A Mahbub[1], Christine L. Le Maitre[1], Sarah L. Haywood-Small[1], Gordon J. McDougall[2], Neil A. Cross[1] and Nicola Jordan-Mahy[1,*]

[1]*Biomedical Research Centre, Faculty of Health and Wellbeing, Sheffield Hallam University, Sheffield, S1 1WB;* [2]*The James Hutton Institute, Environmental and Biochemical Sciences Group, Invergowrie, Dundee Scotland, DD2 5DA*

Abstract: *Background*: Mortality rates for leukemia are high despite considerable improvements in treatment. Since polyphenols exert pro-apoptotic effects in solid tumors, our study investigated the effects of polyphenols in haematological malignancies. The effect of eight polyphenols (quercetin, chrysin, apigenin, emodin, aloe-emodin, rhein, *cis*-stilbene and *trans*-stilbene) were studied on cell proliferation, cell cycle and apoptosis in four lymphoid and four myeloid leukemic cells lines, together with normal haematopoietic control cells.

Methods: Cellular proliferation was measured by CellTiter-Glo® luminescent assay; and cell cycle arrest was assessed using flow cytometry of propidium iodide stained cells. Apoptosis was investigated by caspase-3 activity assay using flow cytometry and apoptotic morphology was confirmed by Hoescht 33342 staining.

Results: Emodin, quercetin, and *cis*-stilbene were the most effective polyphenols at decreasing cell viability (IC_{50} values of 5-22 μM, 8-33 μM, and 25-85 μM respectively) and inducing apoptosis (AP_{50} values (the concentration which 50% of cells undergo apoptosis) of 2-27 μM, 19-50 μM, and 8-50 μM respectively). Generally, lymphoid cell lines were more sensitive to polyphenol treatment compared to myeloid cell lines, however the most resistant myeloid (KG-1a and K562) cell lines were still found to respond to emodin and quercetin treatment at low micromolar levels. Non-tumor cells were less sensitive to all polyphenols compared to the leukemia cells.

Conclusions: These findings suggest that polyphenols have anti-tumor activity against leukemia cells with differential effects. Importantly, the differential sensitivity of emodin, quercetin, and *cis*-stilbene between leukemia and normal cells suggests that polyphenols are potential therapeutic agents for leukemia.

Keywords: Apoptosis, cell cycle, cell proliferation, leukemia, polyphenols.

1. INTRODUCTION

Leukemia affects millions of people worldwide each year, resulting in almost one-third of all cancer deaths [1]. Leukemia is a complex disease affecting all blood cell lineages. Each classification is based, in part, on specific chromosomal and oncogenic rearrangements. Leukemia affects all age groups; it is the most common cancer in children and adolescents [1]. T- and B-cell lymphoblastic leukemia is the most common childhood disease [1, 2], whilst Bcr-Abl-positive chronic myeloid leukemia (CML) is the most common in adults [1, 2]. The treatment regimens for leukemia will depend upon the leukemia type and the patient's age and health. Treatments include chemotherapy, radiotherapy, immuno-therapy and bone marrow transplantation [1, 2]. Newer therapies also being used include tyrosine kinase inhibitors such as Imatinib, which specifically target the constitutively active tyrosine kinase domain of Bcr-Abl fusion gene present in the majority of chronic myeloid leukemia [3]. Despite considerable improvements in tolerance and efficacy of these treatments, the mortality rate of leukemia still remains high [1, 2]. Chemotherapies are by far the most commonly used treatments, however, many are expensive, mutagenic, carcinogenic or teratogenic [2]. Patients often experience considerable side effects, which are so severe that patients sometimes withdraw themselves from treatment, which results in poor prognosis [1, 2]. In addition, patients often fail to get complete disease remission, due to increased occurrence of drug resistance. It is for this reason, that it is important to find new treatments that can improve patient survival rates [1, 2].

*Address correspondence to this author at the Sheffield Hallam University, Faculty of Health and Wellbeing, Sheffield, UK; Tel: 0114 225 3120; Fax: 0114 225 3064; E-mail: N.Jordan-Mahy@shu.ac.uk

These problems with current treatments have led to the search for new compounds for the treatment of leukemia. One area that has received great interest is the use of bio-active agents from natural sources [4-10]. Two groups of bioactive components that have shown potential are the polyphenols and polyacetylenes [4-10]. Epidemiological data has shown that diets rich in polyphenols significantly improve the quality of life and survival rates of patients with a range of chronic diseases, including cancer [11, 12]. Furthermore, these polyphenols are found naturally in a variety of foods and are well tolerated, with few side effects [11-13]. The selected polyphenols used in this study are representative of 3 different classes of polyphenols, which have been previously shown to have anti-proliferative, pro-apoptotic and/or prevent the progression of solid tumors [9, 11-16] and a handful of leukemic cell lines, with the most commonly studied being the human promyleocytic: HL-60 cells [17-21]. The polyphenols investigated include the flavonol (quercetin), flavones (apigenin and chrysin), anthraquinones (emodin, aloe-emodin and rhein); and two stilbene isomers (*cis*-stilbene and *trans*-stilbene) (Table **1**).

Previous work has demonstrated the pro-apoptotic and anti-cancerous activity of polyphenols in a number of solid tumors [11-16] and a selection of leukemic cell lines [17-21]. However, there has not been a comprehensive comparison of the action of polyphenols within a wide range of leukemic cell lines. From previous studies, it is difficult to determine which polyphenols have the greatest potential for the treatment of leukemia. There are no direct comparisons of the IC_{50} values (the concentration which inhibits 50% of cell proliferation) or AP_{50} values (the concentration at which 50% of cells undergo apoptosis) for each polyphenol. Furthermore, it is unclear whether a single polyphenol is affective in all leukemia types; or whether specific polyphenols are only useful in single type or subset of leukemia. For this reason we

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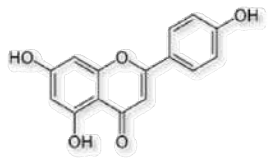
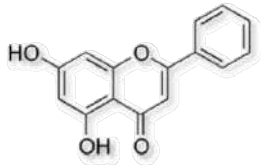
Table 1. The chemical structure and classification of each selected polyphenols.

Classification and Polyphenols Chemical Structure	
Flavonoid (Quercetin),	**Anthraquinone (Emodin)**
Flavonoid (Apigenin)	**Anthraquinone (Aloe-emodin)**
	
Flavonoid (Chrysin)	**Anthraquinone (Rhein)**
	
Stilbene (Cis-stilbene)	**Stilbene (Trans-stilbene)**

This table shows the chemical structure of the flavonoids: flavonol (quercetin) and flavones (apigenin and chrysin); the anthraquinones (emodin, aloe-emodin and rhein); plus the two stilbene isomers (*cis*-stilbene and *trans*-stilbene).

compared the anti-proliferative and pro-apoptotic effects of the 8 polyphenols that have previously shown potential in solid and leukemic cell lines, on a panel of leukemic cell lines that represent the major leukemia types. These included four myeloid (KG-1a, HL-60, THP-1 and K562), three lymphoid (Jurkat, CCRF-CEM and MOLT-3) human leukemic cell lines and one histocytic lymphoma cell line (U937). In addition, for the first time we evaluated the action of these polyphenols on non-tumor hematopoietic stem progenitor cells (CD34+) from cord blood.

The aim of this study was to determine which polyphenols were the most effective at inhibiting cell proliferation and inducing apoptosis in each of the eight leukemic cell lines, whilst having a limited effect on the non-tumor cells. A direct comparison was made of the IC_{50} and AP_{50} values of each polyphenol in each cell line. Furthermore, we determined the action of each polyphenol on cell-cycle progression.

2. MATERIALS AND METHODS

2.1. Leukemia Cell Lines

Four myeloid human leukemia cell lines (HL-60 (Human promyelocytic leukemia) (ATCC: CCL-240, Middlesex, UK), THP-1 (acute monocytic leukemia) (ATCC: TIB-202, Middlesex, UK), K562 (chronic myeloid leukemia) (ATCC: CCL-243, Middlesex, UK) and KG-1a (acute mylogenous leukemia)), three human lymphoid cell lines (Jurkat (peripheral blood T cell leukemia) (ATCC: TIB-152, Middlesex, UK), MOLT-3 (acute lymphoblastic leukemia patient released following chemotherapy) (ATCC: CRL-1552, Middlesex, UK), and CCRF-CEM (acute lymphoblastic leukemia) (ATCC: CCL-119, Middlesex, UK)) and one histocytic lymphoma cell line (U937) (ATCC: CRL-1593.2, Middlesex, UK) together with the non-tumor cord blood (CD34+) cells (Stem cell Technologies, Grenoble, France), were used in this study. All leukemia cell lines except MOLT-3 are p53-deficient, being either null, or containing mutant p53 [22-24]. MOLT-3 cells express wild type p53 [25], but are mutant for PTEN [26]. All cells were tested for mycoplasma contamination using the MycoAlert™ mycoplasma detection kit (Lonza Walkersville, Inc) and were all tested negative throughout the study.

2.2. Culture Conditions

Two million cells per milliliter were seeded in T75cm² flasks (Invitrogen, Paisley, UK) in RPMI 1640 medium (Invitrogen, Paisley, UK) supplemented with 10% (v/v) fetal bovine serum, 1.5mM L-Glutamine and 100 µg/ml penicillin/streptomycin (complete RPMI) and incubated at 37ºC with 5% CO_2.

2.3. CellTiter-Glo® Luminescent Cell Viability Assay

The CellTiter-Glo® Luminescent Cell Viability Assay Kit (Promega, Southampton, UK) was used as a homogeneous method to determine the number of viable cells in culture was based on a quantification of ATP levels. This assay was used to determine the effect of each polyphenols on cellular proliferation in each of the cell lines. Cells were seeded into white 96-well plates (Fisher Scientific, Loughborough, UK) at 2.5 x 10^3 cells per well and treated with each polyphenol dissolved in ethanol: quercetin, apigenin, chrysin, emodin, aloe-emodin, rhein, *cis*-stilbene and *trans*-stilbene (Sigma, Poole, UK) at concentrations between 2 - 500 µM for 24, 48 and 72 h together with ethanol vehicle controls at 0.1 % (v/v) ethanol. All treatments were performed in triplicate, in three independent experiments. Following treatments, cellular proliferation was measured as per manufacturer's instructions. The IC_{50} was determined for each polyphenol in each cell line. This was defined as the treatment concentration at which 50% reduction in cellular proliferation was observed. This was calculated from a linear regression equation of each standard curve for each polyphenol with each cell line. The IC_{25} was also determined in order to provide treatment ranges for apoptosis detection, and cell cycle treatments, but were not used to determine the effectiveness of treatments.

2.4. Cell Cycle Analysis using Propidium Iodide (PI) and Flow Cytometry

The effect of polyphenols on the progression of the cell cycle was studied using flow cytometric analysis using Propidium Iodide (PI) stain. Propidium Iodide emits red fluorescence when intercalated with double stranded nucleic acids. It can be used to quantify the proportion of cells in each phase of cell cycle (G_0/G_1, S and G_2/M); and determine whether cells are accumulated in a specific phase. For cell cycle analysis, cells were seeded in 12 well plates at 0.5 x 10^6 cells per well and treated for 24 h with the IC_{50} concentrations of each polyphenol determined by CellTiter-Glo® assay. Following treatment cells were harvested and centrifuged at 400 g for 5 min. The supernatant was removed, and cells were washed twice in 100 µl cold PBS. Cells were fixed by adding 100 µl of 80% ethanol/H_2O (v/v) and stored overnight at -20°C. Then, cells were washed twice with cold PBS prior to addition of 300 µl of 50 µg/mL PI (Sigma, Poole UK) and 50 µl of 0.1 unit/mL RNase (Sigma, Poole UK). Samples were PI stained overnight at 4ºC and analyzed on the flow cytometer with BD FACS Calibur instrument. Ten thousand events were acquired per sample and the DNA histogram of cell cycle phase was analyzed with FlowJo software using the Waston (pragmatic) equation (Tree Star, Ashland, OR, USA).

2.5. Apoptotic Analysis

Cells were seeded in 12 well plates 0.5 x 10^6 cells per well and treated for 24 h with dose ranges between IC_{25} and IC_{50} for each polyphenol as determined from the CellTiter-Glo®. Apoptosis was assessed using the NucView caspase 3 activity assay (Cambridge Bioscience, Cambridge, UK) and morphological assessment of Hoescht 33342 stained cells (Sigma, Poole, UK).

2.5.1. NucView Caspase 3 Activity Assay by Flow Cytometry

The NucView caspase 3 activity assay is a novel cell membrane permeable fluorogenic caspase substrate designed for detecting caspase 3 activity; which is believed to play a key role in the initiation of cellular events during early apoptosis. Using this method, it was possible to determine the AP_{50} concentrations for each polyphenol in each leukemic cell line. This was defined as the treatment concentration at which 50% of treated cells had undergone apoptosis. Following treatments, 200 µl of each cell suspension was transferred to a flow cytometry tube and 5 µl of caspase 3 activity assay (0.2 mM) (Promega, Southampton, UK) was added. This was incubated for 10 min in the dark, and then each sample was analyzed on the flow cytometer using a BD FACS Calibur instrument (BD, Oxford, UK). Ten thousand events were acquired per sample and the data was analyzed using Flow Jo software (Tree Star, Ashland, OR, USA).

2.5.2. Hoechst 33342 Nuclear Morphological Analysis by Fluorescence Microscopy

Apoptotic cells and nuclear morphology was assessed by fluorescence microscopy following Hoechst 33342 nucleic acid staining. Following polyphenol treatments, cells from each culture well was transferred to eppendorf tubes and centrifuged for 5 min at 400 g at 4ºC. The supernatant was removed, and cells washed in 100 µl PBS. The cells were fixed in 4% (w/v) paraformaldhyde/ PBS and cytospins formed (Shandon Cytospin 3 Centrifuge, Thermo, US). Samples were air dried and then stained in 50 µl of 10 µg/ml Hoescht 33342 staining (Sigma, Poole, UK) for 10 min in the dark. Slides were mounted in immersion oil and examined using a fluorescence microscope (Olympus, BX60, UK). Two hundred cells (live and apoptotic) were counted and the percentage of apoptotic nuclei determined for each sample. Images were captured using LabWorks 4.0 (UVP BioImaging Systems, Loughborough, UK).

2.6. Statistical Analysis

The means and standard deviations (STD) were calculated. Stats Direct software (Stats Direct Ltd, England) was used to test whether data followed a normal distribution using a Shapiro Wilke test. Data which did not follow a normal distribution, was transformed using the logit transformation and statistically analyzed using one way ANOVA and Tukey post hoc tests to investigate significant differences. Results were considered statistically significant when $P \leq 0.05$.

3. RESULTS

3.1. Effects of Polyphenol Treatments on Cell Proliferation in Leukemia Cell Lines

Treatment with polyphenols for 24 h resulted in reduced cell proliferation in all 8 leukemia cell lines to a greater extent than in non-tumor cells (Fig. **1** and Tables **2**, **3**). Using the lowest dose of polyphenols at which there was a significant inhibition on cellular proliferation (Table **2**) and IC_{50} values (Table **3**); it was possible to rank the polyphenols in order of effectiveness. The most effective polyphenols at significantly reducing cell proliferation compared to vehicle controls ($p<0.05$) were emodin, quercetin, and *cis*-stilbene (Fig. **1** and Table **2**). A more moderate affect was shown by apigenin and rhein; and the least effective polyphenols were aloe-emodin, *trans*-stilbene and chrysin. Both lymphoid and myeloid leukemia cell lines were sensitive to emodin, quercetin, and *cis*-stilbene treatment (Fig. **1** and Table **2**). However, it is important to note that each leukemia cell line demonstrated differing sensitivity with the remaining polyphenols. Generally, the lymphoid cell lines were usually more sensitive to polyphenol treatment than myeloid cell lines (Fig. **1** and Tables **2**, **3**).

Emodin consistently gave the lowest IC_{50} values (5-22 µM) (Table **3**), showing a significant effect on cellular proliferation of all leukemia cell lines, with a slightly greater effect on lymphoid than myeloid cells. Emodin also significantly reduced proliferation in the non-tumor cells ($p<0.05$). However, the IC_{50} in the non-tumor cells (~150 µM) was much greater than that seen for all the leukemia cells, demonstrating selectivity towards leukemia cell lines (Fig. **1** and Tables **2**, **3**). Similarly, quercetin had a more potent effect on lymphoid cell line (IC_{50} value 8-20 µM) than myeloid cell lines (IC_{50} 33-155 µM). The least sensitive leukemia cell line to quercetin treatment with an IC_{50} of 155 µM was the acute myelogenous leukemia KG-1a cell line (Fig. **1** and Table **3**). However the human promyelocytic leukemia (HL-60) cell line had a much lower IC_{50} value (8 µM), which was similar to those values seen in lymphoid cells (Fig. **1** and Table **3**). *Cis*-stilbene demonstrated IC_{50} values of 25-85 µM (Fig. **1** and Tables **2**, **3**) and affected both lymphoid and myeloid cells equally (Fig. **1**).

Apigenin and rhein had a moderate effect on cellular proliferation. Apigenin demonstrated a greater effect on the lymphoid cells (IC_{50} 140-195 µM) compared to the myeloid cells (IC_{50} 100-500 µM) (Fig. **1** and Table **3**). Rhein demonstrated a significant decrease in cellular proliferation of all leukemia cell lines and the non-tumor cells ($p<0.05$), with a similar effect seen in both lymphoid and myeloid cell lines (Fig. **1** and Tables **2**, **3**).

Aloe-emodin, chrysin and *trans*-stilbene were the least effective polyphenols on cellular proliferation. Aloe-emodin had IC_{50} values between 180-450 µM; more than ten times higher than emodin. Aloe-emodin, like emodin, showed a greater effect on lymphoid cell lines than myeloid cell lines (Fig. **1** and Tables **2**, **3**). Similarly, chrysin demonstrated comparatively high IC_{50} values, and again was more effective on lymphoid cells (IC_{50} chrysin 128-217 µM) compared to the myeloid cells (IC_{50} 335-500 µM respectively) (Fig. **1** and Table **3**). *Trans*-stilbene had some of the highest IC_{50} values ranging between 109-500 µM. These were much higher than those values found with its isomer, *cis*-stilbene (Fig. **1** and Tables **2**, **3**). Despite the differing effects on the leukemia cells, *trans*-stilbene did not affect the cellular proliferation of the non-tumor cells, until the treatment dose reached 500 µM (Fig. **1**).

3.2. Cell Cycle Accumulation Following Polyphenol Treatments in Leukemia Cells

Treatment of leukemic cell lines at the IC_{50} as determined by CellTiter-Glo® assay of each polyphenol for 24 h significantly induced cell cycle arrest in all leukemia cell lines ($p<0.05$) (Table **4**). There was however no significant arrest in cell cycle in the non-tumor progenitor cells (CD34+) within the IC_{50} treatment ranges used for leukemic cell lines (Table **4**). The phase of cell cycle accumulation varied according to polyphenol treatment and cell line (Table **4**). For example, Jurkat cells demonstrated cell cycle accumulation in S-phase following quercetin, apigenin, rhein, aloe-emodin and *trans*-stilbene treatments ($p<0.05$) (Fig. **2**, Table **4**), whilst cells accumulated in G_0/G_1 phase following chrysin, emodin and *cis*-stilbene treatment ($p<0.05$) (Fig. **2** and Table **4**). A more consistent effect was seen following emodin treatment, which accumulated the cells at G_0/G_1 phase in all leukemia cell lines. Similarly *cis*-stilbene and chrysin also induced cells accumulation at G_0/G_1 phase in 7 out of the 8 leukemia cell lines (Table **4**). Generally, polyphenols appeared to cause G_0/G_1 phase accumulation in most of leukemic cell lines (Table **4**).

3.3. Induction of Apoptosis Following Polyphenol Treatments in Leukemia Cells

All eight polyphenols induced significantly higher levels of apoptosis determined by caspase 3 activity in all leukemia cell lines

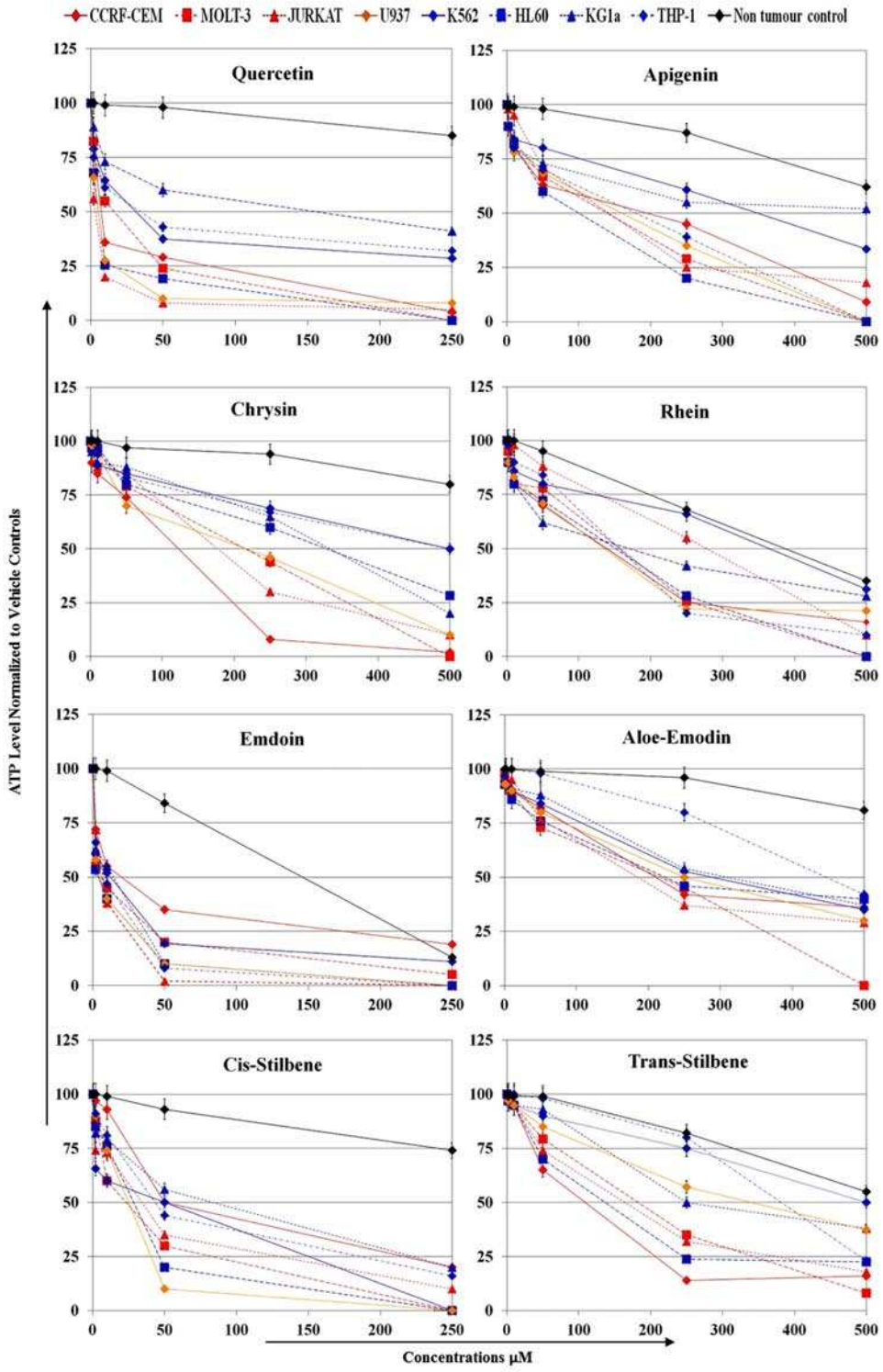


Fig. (1). Effect of eight polyphenols (quercetin, apigenin, chrysin, rhein, emodin, aloe-emodin, *cis*-stilbene and *trans*-stilbene) on cellular proliferation of three lymphoid leukemia (CCRF-CEM, MOLT-3, JURKAT; red lines), one histocytic lymphoma (U937; orange lines), four human myeloid leukemia cell lines (K562, HL-60, KG-1a , THP-1; blue lines), and one non-tumor normal progenitor cells (CD34+; black line). This was evaluated by CellTiter-Glo® assay. Cells were treated with 0, 2, 10, 50, 250 µM of quercetin, emodin, *cis*-stilbene; and with 0, 2, 10, 50, 250, 500 µM of apigenin, chrysin, rhein, aloe-emodin, *trans*-stilbene for 24 h. Data was normalized to the vehicle control which was assigned 100% cell viability. The data is expressed as mean ± STD (three independent experiments, each in triplicate). The statistical significance was determined by comparison with the vehicle control, statistical significance was set at $p<0.05$ and determined by one way ANOVA and Tukey post-hoc test. Statistical results are summarised in Table **2** which shows the lowest dose that induced significant inhibition compared to vehicle control. All concentrations above these points were also statistically significant. The IC_{50} for each polyphenol in each cell line were determined and shown in Table **3**.

Table 2. The lowest dose of polyphenols that induced a significant decrease in cellular proliferation compared to the vehicle controls, $p<0.05$. Polyphenol treatments were: 0, 2, 10, 50, 250, 500 μM for 24 h.

Cell Types			The lowest dose of polyphenols (μM) at which there was a significant inhibition of cell proliferation compared to the vehicle control.							
			Quercetin	Apigenin	Chrysin	Rhein	Emodin	Aloe - Emodin	Cis- Stilbene	Trans-Stilbene
Cell lines	Lymphoid leukaemia	JURKAT	2	50	50	50	2	50	2	50
		CCRF-CEM	2	10	50	50	2	50	10	50
		MOLT-3	2	50	50	50	2	50	2	50
	Myeloid leukaemia	HL60	2	10	50	50	2	50	2	50
		THP-1	2	10	250	50	2	250	2	250
		K562	2	10	250	50	2	50	2	50
		KG1a	10	10	250	50	2	50	2	50
	Histocytic lymphoma	U937	2	10	50	50	2	50	2	50
Peripheral blood cells	Non-tumour control cells	CD34+	250	500	500	250	50	500	250	250

The polyphenols were ranked in order of activity with respect to significant reduction of cellular proliferation in lymphoid cells (emodin = quercetin > *cis*-stilbene > apigenin > rhein = *trans*-stilbene = aloe-emodin = chrysin); and in myeloid cells (emodin = *cis*-stilbene ≥ quercetin > apigenin > rhein > aloe-emodin = *trans*-stilbene > chrysin). Note that the treatment doses that caused significant inhibition of cellular proliferation in all leukemic cell lines were much lower than in the non-tumor cells (CD34+)**.** Due to the wide range of concentrations used and the number of cell lines investigated, it was not possible to indicate significance levels on Fig. (**1**), and thus, Table **2** indicates the lowest dose of polyphenol at which significance was obtained for each cell line, providing the statistical analysis for Fig. (**1**).

Table 3. The IC_{50} values responsible for 50% inhibition of cellular proliferation in each leukemic and non-tumor control cell line following 24 h treatment with each polyphenols.

Cell Types			Polyphenols IC_{50} in μM							
			Quercetin	Apigenin	Chrysin	Rhein	Emodin	Aloe - Emodin	Cis- Stilbene	Trans-Stilbene
Cell lines	Lymphoid leukaemia	JURKAT	10	143	180	277	9	185	38	163
		CCRF-CEM	10	195	128	140	22	211	53	109
		MOLT-3	20	140	217	158	8	220	25	180
	Myeloid leukaemia	HL60	8	100	328	150	5	225	32	135
		THP-1	37	180	500	158	10	450	45	380
		K562	33	350	340	380	13	309	53	500
		KG1a	155	500	335	169	15	310	85	250
	Histocytic lymphoma	U937	8	160	217	135	7	250	30	340
Peripheral blood cells	Non-tumour control cells	CD34+	>500	>500	>500	380	150	>500	>500	500

This was determined by CellTiter-Glo® Luminescent assay. The polyphenols were ranked in order of activity with respect to inhibition of 50% proliferation in lymphoid cells (emodin = quercetin > *cis*-stilbene > apigenin > *trans*-stilbene ≥ chrysin = rhein > aloe-emodin); and in myeloid cells (emodin = *cis*-stilbene ≥ quercetin > apigenin = rhein > aloe-emodin = *trans*-stilbene = chrysin). In Non-tumour cells (CD34+), did not reach 50% inhibition until the polyphenol treatments excessed 500μM, the only exceptions were emodin and rhein. Note that the highest doses of aloe-emodin, chrysin, rhein and *trans*-stilbene would be clinically impractical, while quercetin emodin, *cis*-stilbene had much lower doses and thus are potentially more clinically useful.

Table 4. The effect of polyphenol treatment on the cell cycle progression in myeloid and lymphoid cell lines.

Cell lines		Percentage of cells in all phases of cell cycle	The percentage of cells in the phases of cell cycle at which there was a significant accumulation of cells when compared to vehicle controls after treatment with IC_{50} dose of each polyphenol following 24h.							
		Vehicle Control	Quercetin	Apigenin	Chrysin	Rhein	Emodin	Aloe - Emodin	Cis- Stilbene	Trans-Stilbene
Lymphoid leukaemia	JURKAT	G0/G1 = 52.13% S = 36.06% G2/M = 10.01%	S = 51%	S = 49%	G0/G1 = 65%	S = 45.70%	G0/G1 = 60%	S = 49%	G0/G1 =65%	S = 47%
	CCRF-CEM	G0/G1 = 41.9% S = 47.2% G2/M = 7.8%	S = 51.4%	G0/G1 =52.2%	G0/G1 =49.4%	G0/G1 =54%	G0/G1 =50%	G0/G1 =49%	G0/G1 =51.1%	G0/G1 =49.1%
	MOLT-3	G0/G1 = 64.5% S = 24.8% G2/M = 7.5%	G2/M =19%	S =35%	G0/G1 =69.3%	G0/G1=72%	G0/G1 =70%	G0/G1 = 68%	G2/M = 15%	G0/G1 = 69%
Myeloid leukaemia	HL60	G0/G1 = 56.8% S = 33.6% G2/M = 8.45%	G0/G1 =66.2%	G0/G1=67%	S =45.5%	G0/G1=65%	G0/G1 =67.7%	S =35%	G0/G1 =70.9%	S =41.9%
	THP-1	G0/G1 = 42.3% S = 31.1% G2/M = 26.4%	G0/G1 =52%	G2/M =32%	G0/G1 =52.1%	G0/G1 =47%	G0/G1= 50.8%	G0/G1 =52%	G0/G1=50%	G0/G1 =53%
	K562	G0/G1 = 53.7% S = 32.06% G2/M = 13.6%	G0/G1=60%	S =50.2%	G0/G1 =59%	S =48%%	G0/G1 =66%	G2/M =20%	G0/G1 =64%	G2/M =24.5%
	KG1a	G0/G1 = 44.6% S = 34.6% G2/M = 18.7%	G0/G1=52%	S = 50%	G0/G1 =50%	G0/G1 =49%	G0/G1 =52.9%	G0/G1 =50%	G0/G1 =51%	G2/M =23.7%
Histocytic lymphoma	U937	G0/G1 = 44.03% S = 40.1% G2/M = 19.1%	G2/M =25%	G0/G1 =59%	G0/G1 =70%	G0/G1 =55%	G0/G1 =60%	G0/G1 =53%	G0/G1 =64%	G0/G1 =62%
Non-tumour control cells	CD34+	G0/G1 = 53% S = 25.8% G2/M = 20.7%	No Arrest <50μM	No Arrest ≤250μM	No Arrest <250μM	No Arrest <50μM	No Arrest <50μM	No Arrest <500μM	No Arrest ≤250μM	No Arrest ≤500μM

The cell cycle phase was assessed by flow cytometric analysis of propidium iodide (PI) stained cells, and the percentage of cells accumulation in each phase of cell cycle (G_0/G_1, S, G_2/M) was determined from the DNA histograms of each sample analysing by FlowJo software using Waston (pragmatic) equation**.** The data shows the phases of cell cycle in which each cell type was significantly accumulated when compared with the vehicle control, when treated for 24 h with IC_{50} concentration for each polyphenol, as determined by CellTiter-Glo® assay ($p<0.05$). The table shows the percentage of cells in each phases of cell cycle at which there was a significant accumulation. No significant arrest in cell cycle was observed in the non-tumor progenitor cells (CD34+) within the IC_{50} ranges used to treat the leukemic cell lines.

compared to the non-tumour cells (*p<0.05*) (Fig. **3** and Table **5**). Emodin, quercetin, *cis*-stilbene and apigenin were the most effective polyphenols at inducing apoptosis with AP_{50} values ranging between 2-27 μM, 19-50 μM, 8-50 μM, 35-150 μM, respectively, in all leukemia cell lines (Table **6**). The leukemia cell lines demonstrated differing sensitivity to the polyphenols; Jurkat lymphoid cells were most greatly affected, whilst THP-1 myeloid cells were the least affected to all polyphenols treatments (Fig. **3**).

Quercetin, apigenin, emodin, aloe-emodin and chrysin demonstrated a greater toxicity towards lymphoid leukemia cell lines than myeloid leukemia cell lines (Fig. **3** and Tables **5, 6**). In contrast, rhein, *cis*-stilbene and *trans*-stilbene demonstrated similar sensitivity to both myeloid and lymphoid cell lines. Some cell lines were more resistant to polyphenol treatment. The THP-1 myeloid cell line was only sensitive to emodin, rhein and apigenin treatment (Fig. **3** and Tables **5, 6**); whilst the myeloid cell lines (K562 and KG-1a) were only sensitive to apigenin treatment (Fig. **3** and Table **6**).

Morphological assessment of apoptosis by Hoechst 33342 staining confirmed the patterns of apoptosis induction seen in caspase 3 activity assays, although the AP_{50} values determined using this method were higher demonstrating that the progression to the later stages of apoptosis required a greater treatment dose (Fig. **4** and Table **6**).

4. DISCUSSION

Over the past 10 years, researchers have confirmed that dietary polyphenols are capable of inhibiting cell proliferation, inducing cell cycle arrest and apoptosis in a number of solid tumor cell lines [11-16], however there has not been a direct comparison of the effect of polyphenols on leukemia cell lines and non-tumor cells. Here, we directly compared the effect of eight polyphenols (quercetin, apigenin, chrysin, rhein, emodin, aloe-emodin, *cis*-stilbene and *trans*-stilbene) on four lymphoid and three myeloid leukemia cell lines; one histocytic leukemia cell line; and the non-tumor blood progenitor cells (CD34+). The effects of these polyphenols were shown to be greater in leukemia cells compared to non-tumor blood progenitor cells (CD34+). When non-tumor cells were treated with quercetin and *cis*–stilbene, chrysin, apigenin

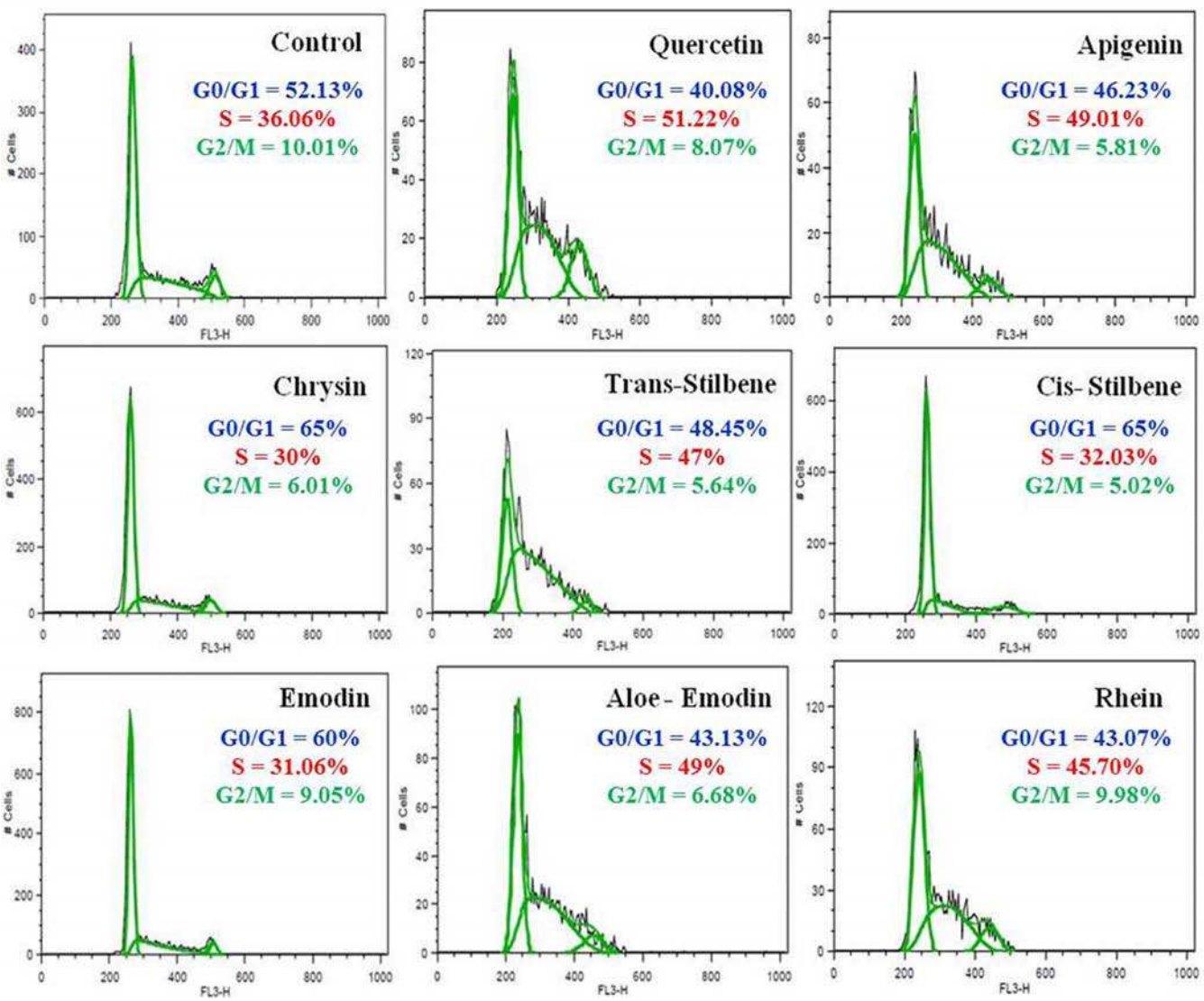


Fig. (2). An example of the cell cycle phases (G_0/G_1, S, G_2/M) for the acute T cell leukemia (Jurkat) cells after treatment with IC_{50} concentration of each polyphenol following 24 h as determined by CellTiter-Glo® assay. The percentage of cells in each phase was analyzed with Flow Jo software using Watson pragmatic model. Each polyphenol caused a significant accumulation of cells in cell cycle comparing to the vehicle control. Quercetin, apigenin, *trans*-stilbene and aloe-emodin and rhein significantly induced accumulation in S-phase ($p<0.01$), in contrast emodin, *cis*-stilbene and chrysin significantly induced accumulation in G_0/G_1 phase ($p<0.01$).

and aloe-emodin, there were no significant decrease on cellular proliferation until the treatment concentration increased to 250-500 μM. There was a significant decrease on proliferation of non-tumor cells when treated with ≥250 μM of emodin, rhein and *trans*-stilbene; however this is 5-10 times higher than the IC_{50} values reported for all leukemia cells (Fig. **1** and Tables **2**, **3**). Consequently, we have shown that each of the polyphenols caused a decrease in proliferation in all leukemia cell lines and can be ranked according to their effectiveness: emodin > quercetin > *cis*-stilbene > apigenin ≥ rhein > aloe-emodin ≥ *trans*-stilbene ≥ chrysin. However, it is important to note that this ranking did vary between individual cell lines (Table **3**).

Emodin was the most effective polyphenol at reducing cellular proliferation. It was by far the most effective of the anthraquinones investigated. The structural differences between the anthraquinones are slight and, indeed, emodin and aloe-emodin have the same structural formula ($C_{15}H_{10}O_5$), although the orientations of the functional groups vary. The IC_{50} values for emodin (5-22 μM) were the lowest of all the studied polyphenols; and were comparable with those previously reported in squamous cell carcinoma (SCC-4) cells [27]. Emodin was shown to consistently induce accumulation of cells at G_0/G_1 phase in all leukemia cell lines, and induced 50% apoptosis in 5 of the 8 leukemia cell lines (Jurkat, MOLT3, HL-60, THP-1 and U937). This is consistent with previous studies in which emodin induced apoptosis in HL-60 [28] and SCC-4 [27] cells.

Quercetin was also a potent polyphenol, with IC_{50} value ranging between 8-33 μM and induction of apoptosis with AP_{50} value ranging between 19-50 μM. Quercetin was the most effective of the flavonoids tested and was routinely 5-10 times more potent than apigenin and chrysin. The IC_{50} values noted are at the lower end of values previously reported (20-278 μM), in breast (MDA-MB-231 and MDA-MB-453 [29, 30], MCF-7 [31]), cervical (HeLa) [32-34], liver (HepG2) [35], lung (A-549) [36] and leukemia cell lines (HL-60 and K562) [17, 37]. Lymphoid cell lines were more susceptible to quercetin treatment than myeloid leukemia cells. The only exception being the promyelocytic leukemia cells (HL-60), which

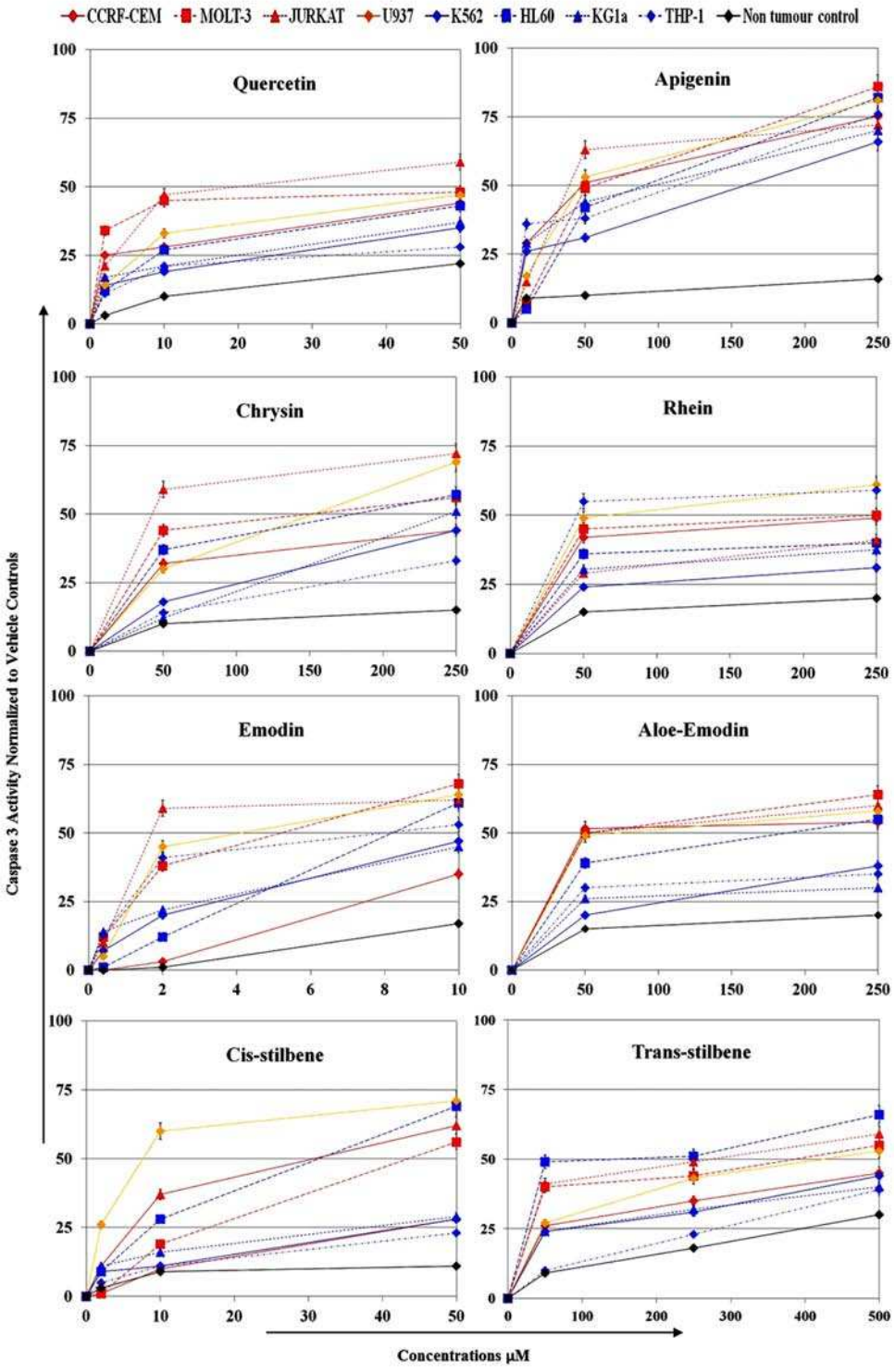


Fig. (3). Effect of eight polyphenols (quercetin, apigenin, chrysin, rhein, emodin, aloe-emodin, two *cis*-stilbene and *trans*-stilbene) on apoptosis of three lymphoid leukemia (CCRF-CEM, MOLT-3, and JURKAT; red lines), one histocytic lymphoma (U937; orange lines), four human myeloid leukemia cell lines (K562, HL-60, KG-1a , THP-1; blue lines) and the non-tumor normal progenitor cells (CD34+; black line). Apoptosis was assessed using a caspase 3 activity assay and analyzed by flow cytometry. Cells were treated with range of concentrations for each polyphenol for 24 h and the range of IC25 and IC_{50} as determined by CellTiter-Glo® assay. The treatment concentrations for emodin were 0, 0.4, 2, 10, 50 µM, for quercetin and *cis*-stilbene were 0, 2, 10, 50 µM; and for apigenin, chrysin, aloe-emodin, rhein and *trans*-stilbene were 0, 10, 50, 250 µM. All data was normalized to the vehicle-only control, which was assigned a 0% apoptotic level. The data is expressed as mean ± STD (three independent experiments, each in triplicate). The statistical significance was determined by comparison with the vehicle control, statistical significant was set at $p<0.05$ and determined by one way ANOVA and Tukey post-hoc test. Statistical results are summarised in Table **5** which shows the lowest dose that induced significant inhibition compared to vehicle control. All concentrations above these points were also significant. The AP_{50} for each polyphenol in each cell line were determined and shown in Table **6**.

Table 5. The lowest dose of polyphenols which induced significant induction of caspase 3 activity, compared to the control ($p<0.05$).

Cell Types			The lowest dose of polyphenols (μM) at which there was a significant induction of apoptosis compared to the vehicle control.							
			Quercetin	Apigenin	Chrysin	Rhein	Emodin	Aloe - Emodin	Cis- Stilbene	Trans-Stilbene
Cell lines	Lymphoid leukaemia	JURKAT	2	50	50	50	2	50	10	50
		CCRF-CEM	2	10	50	50	10	50	50	50
		MOLT-3	10	50	50	50	2	50	10	50
	Myeloid leukaemia	HL60	10	50	50	50	10	50	10	50
		THP-1	10	10	250	50	2	50	50	250
		K562	10	10	250	50	2	50	50	50
		KG1a	10	10	250	50	2	50	10	50
	Histocytic lymphoma	U937	2	50	50	50	2	50	2	50
Peripheral blood cells	Non-tumour control cells	CD34+	50	250	250	50	10	250	250	250

Apoptosis was assessed by caspase 3 activity assay. The polyphenols were ranked in order of activity with respect to significant induction of apoptosis in lymphoid cells (emodin = quercetin ≥ *cis*-stilbene > apigenin > rhein = *trans*-stilbene = aloe-emodin = chrysin); and in myeloid cells (emodin > quercetin > *cis*-stilbene = apigenin > rhein = aloe-emodin ≥ *trans*-stilbene > chrysin). Note that the treatment doses which caused significant induction of apoptosis in all leukemic cell lines were much lower than of the non-tumor cells (CD34+). Due to the wide range of concentrations used and the cell lines investigated, it was not possible to indicate significance levels on Fig. (**3**) and thus Table **5** provides the lowest doses of polyphenol at which significance was obtained.

Table 6. The AP_{50} values responsible for 50% induction of apoptosis, determined by: Caspase 3 activity assay (C3) and Hoechst 33342 staining (Hoe).

Cell Types			Polyphenols AP_{50} in μM															
			Quercetin		Apigenin		Chrysin		Rhein		Emodin		Aloe - Emodin		Cis- Stilbene		Trans-Stilbene	
			C3	Hoe	C3	Hoe	C3	Hoe	C3	Hoe	C3	Hoe	C3	Hoe	C3	Hoe	C3	Hoe
Cell lines	Lymphoid leukaemia	JURKAT	19	25	35	90	40	30	>250	>500	2	9	50	130	31	50	250	310
		CCRF-CEM	50	50	50	100	250	40	200	250	>10	>500	50	150	>50	>500	>500	200
		MOLT-3	50	50	50	125	140	38	200	265	5	9	50	150	44	50	350	400
	Myeloid leukaemia	HL60	50	50	84	129	175	75	>250	>500	8.5	6	185	190	31	49	40	50
		THP-1	>50	>500	110	220	>250	>500	50	60	7.8	10	>250	283	>50	>500	>500	>500
		K562	>50	205	150	190	>250	>500	>250	>500	>10	>500	>250	500	>50	410	>500	460
		KG1a	>50	125	89	235	>250	>500	>250	>500	>10	>500	>250	360	>50	350	>500	360
	Histocytic lymphoma	U937	50	50	45	130	150	32	60	140	4	27	50	195	8	20	200	225
Peripheral blood cells	Non-tumour control cells	CD34+	>50	>500	>250	>500	>250	>500	>250	>500	>10	>500	>250	>500	>50	>500	>500	>500

The polyphenol were ranked in order of induction of 50% apoptosis in lymphoid cells (quercetin ≥ emodin = *cis*-stilbene > apigenin > aloe-emodin > chrysin ≥ rhein > *trans*-stilbene). The HL-60 human promyelocytic leukemia cell line was the only myeloid cell to reach an AP_{50}. The non-tumour cells (CD34+) did not reach 50% apoptosis with any of the treatment doses investigated. THP-1, K562 and KG-1a myeloid cell lines were the most resistant cell lines, although did they reached an AP_{50} with apigenin treatment.

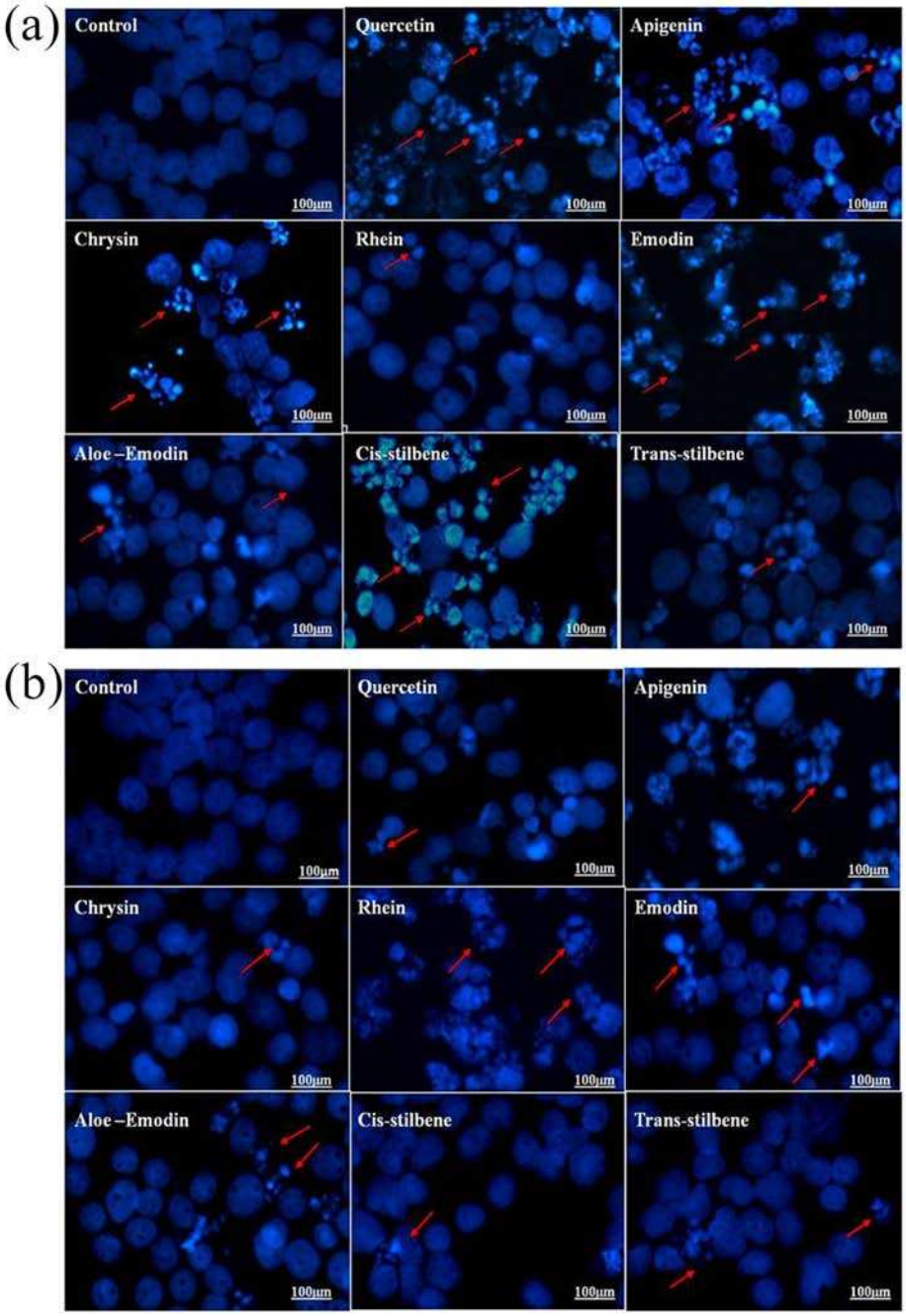


Fig. (4). A typical example of morphological assessment of apoptosis using Hoechst 33342 nuclear staining, after treatment with the IC_{50} concentration for each polyphenol for 24 h: (**A**) Shows the Jurkat lymphoid leukemia cells, which were one of the most sensitive cell lines to polyphenol treatment and (**B**) Shows THP-1 myeloid leukemia cells, which were one of the most resistant cell lines to polyphenol treatment. Apoptotic cells were identified by their irregular shape, intensely stained nuclei, cell shrinkage, blebbing and chromatin condensation and the formation of apoptotic bodies. Scale bar = 100 μm. Arrow indicates examples of apoptotic cells.

showed the same level of sensitivity as lymphoid cells. Quercetin demonstrated a differential induction of apoptosis in each leukemia cell line although the AP_{50} values were consistently low. Previously, quercetin has been reported to induce apoptosis in a range of solid tumors, *via* a caspase 3-dependent mechanism [29, 31 38], and in HL-60 cells *via* decreased PI3K/AKT pathway activity [39]. However, there are no reported AP_{50} values for these studies. Quercetin was found to have a differential effect on the cell cycle in myeloid and lymphoid cell lines. Inducing accumulation of cells at G_0/G_1 phase in all myeloid cell lines, and either S-phase (Jurkat and CCRF-CEM) or G_2/M phase (U937 and MOLT3) accumulation in the lymphoid cell lines (Table **4**). This varied effect of quercetin has been previously observed, where it induced accumulation in S-phase in breast cancer (MCF-7) [31] and in G_2/M phase in cervical cancer (HeLa) cell lines [32, 33]. Together with our study, this suggests that quercetin causes differential effects on cell cycle

dependant on cell type, even in comparatively similar leukemia cell lines. This may reflect expression of different molecular target in myeloid and lymphoid cell lines; or a differential effect on the same pathway in different cell lineages.

Cis-stilbene was much more effective than its isomer *trans*-stilbene in all leukemia cell lines. This is reflected in IC_{50} values for *cis*-stilbene (25-85 μM) and *trans*-stilbene (109-500 μM); however, these values were considerably higher than those previously reported in solid tumors [40, 41]. Very few studies have investigated the effects of stilbenoids on cell cycle. *Cis*-stilbene has been reported to induce cells accumulation in G_2/M phase in the lung cancer cell line (A549) [42], and in S-phase in one leukemia cell line (HL-60) [19]. Our results have shown that *cis*-stilbene consistently caused cell accumulation at G_0/G_1 phase in 7 of the 8 cell lines including the HL-60 cell line. This contrasts with the finding that HL-60 cells when treated with the stilbene derivate 3, 3', 4, 4', 5, 5'-hexahydroxystilbene induced S-phase accumulation [19]. A less consistent effect was found with *trans*-stilbene treatment, which caused cell accumulation at different phases of cell cycle in all leukemia cell lines. Both *cis*- and *trans*-stilbene induced caspase-3 activity of early apoptosis and morphological changes characteristic of late apoptosis in the majority of leukemia cell lines. *Cis*-stilbene was able to induce apoptosis in three lymphoid cell lines (Jurkat, MOLT3, and U937) with AP_{50} values ranging between 20-50 μM, the remaining cell lines were more resistant, and did not reach 50% apoptosis even when treated with a maximal treatment dose (500μM). Similarly, *trans*-stilbene induced apoptosis in the same three lymphoid cell lines, however the AP_{50} values were much higher (40-460 μM), there was also a similar resistance to treatment in the remaining lymphoid cell lines. *Cis*-stilbene did not show any significant effect on proliferation of the non-tumor cells, however, *trans*-stilbene did, but only at high treatment concentration in excess of 250 μM. Previous work has shown that stilbenoids can inhibit cell proliferation and induce apoptosis in lung (A549) [42, 43], prostate (DU145 and PC3), breast (BT-549), colon (HT-29) [40, 41] and one leukemia (HL-60) [19, 20] cell line. *Trans*-stilbene had a reported IC_{50} values of 25-98 μM at 24 h in two lung cancer cell lines (A549 and CH27) [43]. A direct comparison of *cis*-stilbene and *trans*-stilbene in lung (A549) [42] and leukemia (HL-60) [19] cell lines, demonstrated that *cis*-stilbene was more effective than *trans*-stilbene with IC_{50} values of 0.03 μM and 6.25 μM, respectively, at 24 h [19, 42]. This supports the finding of this study that *cis*-stilbene is more potent than *trans*-stilbene in the treatment of leukemia cells. However, the reason for this difference is not clear, but may be related to the stability of the *trans*- and *cis*-isomers in culture.

A moderate effect was seen in leukemia cells treated with apigenin, with IC_{50} values between 100-500 μM. However, in other cell types lower IC_{50} values have been reported, including 36 μM in human cervical cancer cells (HeLa) [44] and 70 μM in colorectal cancer cells (SW480, HT-29 and Caco-2) following 24 h treatments [45] suggesting differential activity within tumour types. In addition, apigenin induced variable effects on cell cycle, which was dependant on the cell lines investigated. This phenomena has also been seen in solid tumor cell lines, where apigenin induced G_0/G_1 arrest in human cervical cancer (HeLa) cells [44] and G_2/M arrest in human colon carcinoma (SW480, HT-29 and Caco-2) [45]. Apigenin was shown to induce apoptosis in all leukemia cell lines, with AP_{50} values ranging between 35-130 μM in lymphoid cell lines and 84-235 μM in the myeloid cell lines. In contrast to quercetin, apigenin was capable of inducing both an increase in caspase 3 indicating early apoptosis, plus morphological evidence of late apoptosis, in all leukemia cells lines; including the KG-1a and K562 cells which were resistant to emodin, quercetin and *cis*-stilbene treatment. This pro-apopotic action of apigenin has been previous demonstrated in MDA-MB-453 breast cells [30]. The other polyphenols investigated; rhein, chrysin, aloe-emodin demonstrated a low potency and thus are unlikely to be of clinical use in leukemia treatment. Similar low potency has also been shown in solid tumors, for example the reported IC_{50} for chrysin in solid tumor cell lines are between 40 and 100μM [46-48].

Within all the polyphenols agents tested, the leukaemia cells were more sensitive than the CD34+ non-tumour cells. Interestingly, the proliferation rates and percentages of G_0/G_1 population were comparable in all untreated leukaemia and non-tumour control cells, suggesting sensitivity rates were not related to rates of proliferation. The order of sensitivity within the leukaemia cells was shown to be dependent on the polyphenol investigated. For example, U937 cells were one of the most affected cell lines when treated with quercetin, emodin and *cis*- stilbene, however they were the were least affected cell line when treated with apigenin. This demonstrates that no single polyphenol is active on all cell lines and that specific polyphenols should be selected for each type of leukaemia.

The cell cycle arrest data showed predominately G_0/G_1 arrest, however some treatments arrested cells in S-phase and G_2M. It is well known that cell cycle is regulated by the coordinated activity of family of protein kinases: cyclin-dependent kinase (CDKs), cyclins and CDK inhibitors (CDKIs) [49]. Cell-cycle can be arrested *via* protein kinase inhibitors (CDKIs), such as $p21^{waf1}$ and $p27^{kip1}$, upon binding to cyclins and CDK complexes and indeed modulation of their activities could be possible targets for the polyphenols. The stage of cell cycle arrest induced by phenolic agents can indicate the molecular mechanisms of action. For example it is well known that cells arrested in G_1 phase can be *via* inhibition of CDK4 and/or CDK6 [49]. S-phase arrest can be caused by inhibition of Cyclin A and Cyclin E through the activation of p21 (*via* p53 in the presence of DNA damage) and p27 (activated by Transforming Growth Factor of β (TGF- β)) [49]. Arrest of cells in G_2/M phase can be caused by inactivation of cyclin B1 with Cdc2 kinase activity through p53 activation [49].

Here, we demonstrated that the majority of polyphenols investigated induce G_0/G_1 arrest, suggesting that they may inhibit CDK4 and/or -6, however this requires confirmation. Hur, *et.al* (2004) showed that Jurkat cells and T lymphocytes stimulated with rosmarinic acid induce $p56^{lck}$ (Lck) protein kinase-dependant apoptosis, through the mitochondrial pathway [50]. $P56^{lck}$ is a lymphoid-specific protein tyrosine kinase and is usually expressed on T lymphocytes [50]. This may explain why the lymphoid cell lines were more sensitive than myeloid cell lines. In addition, recent investigations showed that polyphenols such as the flavanoids (apigenin and quercetin) can act as a $p56^{lck}$ (Lck) protein kinase inhibitors [50, 51]. As $p56^{lck}$ is an essential regulator of the cell cycle; modulation of this kinase could lead to the G_0/G_1 arrest. However, further investigation is essential to determine the molecular mechanisms of each polyphenol.

It is well established that tumor suppressor gene p53 has a role in the regulation of the cell cycle, as well as in the initiation of apoptosis. However the majority of our cell lines were either null or mutated for p53, with the exception of MOLT3 which express wild type p53 [22-25]. MOLT 3 cells however, display PTEN mutations, which results in constitutive activity of AKT [26]. p53 induces Bax, which leads to activation of the intrinsic apoptotic pathway. AKT promotes pro-apoptotic BAD to be sequestered. Therefore a lack of p53 or PTEN both lead to an insensitivity to apoptosis with respect to the intrinsic pathway [52]. This suggests that the p53 status does not influence the effect of polyphenol treatment in this study.

To determine whether the effects of these polyphenols *in vitro* are relevant to their clinical use, it is essential also to consider their bioavailability and whether these treatment concentrations are achievable in plasma. It has been suggested that physiological concentrations of plasma metabolites will not exceed 10 μM [53-55]. Our study has shown that quercetin, emodin and *cis*- stilbene

induced significant affects at low doses (between 2 to 10 μM) following 24 h of treatment in most of leukemic cell lines. The data available on bioavailability of polyphenols however is still limited, but there is evidence that quercetin obtained from plant products can result in micromolar concentrations in blood plasma [54, 56], supporting the idea that *in vivo* effects may be possible, through diet. However, quercetin has a reported plasma half-life of 11–28 h; with a 50-100 mg dose causing a plasma concentration of 0.75–1.5 μM in plasma [53-56]. This is further complicated as abundant dietary polyphenols do not necessarily have the best bioavailability profile [53, 55] and they are extensively metabolized by intestinal and hepatic enzymes and microflora [53, 57]. The absorption of polyphenols depends primarily on their chemical structure, and molecular size as well as the degree of glycosylation, esterification, and polymerization with other polyphenols [53, 55, 57, 58].

In conclusion, we have shown that the effectiveness of polyphenols varied depending on the leukemia cell lineage (lymphoid vs. myeloid) and in some cases within the cell lines from the same lineage. We have shown that myeloid cell lines (K562 and KG-1a) were particularly resistant even to the most active polyphenols. This suggests that the molecular mechanism of action of the polyphenols may vary in each cell line and this requires further investigation. Furthermore, we have demonstrated that polyphenols with similar molecular structures such as emodin and aloe-emodin, and even *cis*- and *trans*-stilbene do not have the same effect on leukemia cells. These findings suggest that polyphenols have anti-tumor activity against leukemia cells with differential effects. The observed differential sensitivity between leukemia and normal cells suggests that polyphenols have potential in treatment of leukemia. The most potent polyphenols are emodin, quercetin, and *cis*-stilbene; these polyphenols may have potential in treating leukemia.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

AP_{50} = the concentration which 50% of cells undergo apoptosis.

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