Anti-Cancer Effect of Aloe Emodin on Breast Cancer Cells, MCF-7

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Abstract- **Phytochemicals of some plants are believed to have natural anti-proliferative properties to various cancer cells. Thus, they might have the potential as alternative choice for contemporary treatment as the latter are usually associated with many unpleasant side effects. The aim of this study is to investigate the possible anti-cancer effect of aloe emodin (AE; 1,8-Dihydroxy-3-hydro-xymethyl-anthraquinone) on estrogen-positive breast cancer cells, MCF-7. We were able to demonstrate the efficiency of AE, an antraquinone derivatives which are present in Aloe Vera leaves, in limiting the proliferation effect of MCF-7 cells in a dose and time dependent manner using WST-1 assay. Our preliminary result suggests that AE could be a promising natural candidate for future pharmacological study, targeting in breast cancer prevention strategies.**

Keywords—Aloe Vera, aloe emodin, breast cancer MCF-7 cells, anti-cancer, WST-1

I. INTRODUCTION

In general, medicinal herbs had been used extensively as an alternative to modern medicine. Healing and medicinal herbs can be found originated from different countries such as Jamaica [1], America [2], China [3] and Russia [4]. In addition, there are even healing herbs that can cater specifically for women [5].

The prospective of some medicinal herbs and natural plants to stimulate tumor growth arrest and death has recently become attractive opportunity to many researchers. The phytochemicals found in some of plants were believed to have the potential as an alternative choice for contemporary treatment that usually associated with many unpleasant side effects.

The aim of this study is to investigate the anti-cancer activity of aloe emodin (AE; 1, 8-Dihydroxy-3-hydro-xymethyl-anthraquinone) on estrogen-positive breast cancer cells, MCF-7. The next section discusses (i) literature review, (ii) methodology, (iii) results and discussion and (iv) conclusion.

II. LITERATURE REVIEW

A. Tamoxifen and Breast Cancer

The role of tamoxifen (TAM) as a treatment for women with hormone receptor positive breast cancer is well documented. Since estrogen is necessary in carcinogenesis of hormone-dependant cancer, the anti-estrogen properties of TAM has made this drug as a standard regimen for the management of estrogen positive breast cancer [6]. However, due to frequent and critical problems arising in tamoxifen-resistant, the research on other alternative natural source has been highlighted recently [7-10]. In this study, we have concurrently included TAM as a positive control in our proliferation assay. The half maximal inhibitory concentration or IC_{50} on MCF-7 for both of AE and TAM were investigated.

B. Direct Measuring Viability of Cells

The very first step of screening any potential medicinal herbs or natural plant for their anti-cancer activity is by determining the viability of cells in culture after treating with the respective active compounds. Generally, there are two modes of choices which technically can be direct or indirect procedures [11]. The direct option can be achieved by actual counting of the living cells after exposing the single cell with a particular stain for example with trypan blue [12], eosin-nigrosin [13] or propidium iodide, PI [14].

The principle of this assay is based on the ability of viable cell to reject certain dye due to its intact cell membrane. Therefore, in trypan blue exclusion method, a viable cell will have a clear cytoplasm while a nonviable cell will take up the blue color [12]. The numbers of stained and unstained cells will be measured using hemacytometer and microscope. Electronic counter system is also integrated with this technique as to accelerate and accurately measuring the viable cells. Evaluation using flow cytometry can be carried out using the fluorescent dye [14]. We have included some literatures on cytotoxicity assessment of AE using this direct technique as summarized in Table I and II.

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TABLE I. CELL VIABILITY ASSAY USING TRYPAN BLUE EXCLUSION TEST

Type of Cell Line	Source of Cell Line	Cell Density	Hours of Treatment (Hours)	Effect of AE	Authors
U937	Human monoblastic leukemia	1 x 10^5 cells/ml	24	10µM reduced the proliferation to 37.8%	[15]
				50µM reduced the proliferation to 52.1%	
			48	10µM reduced the proliferation to 31.1%	
				50µM reduced the proliferation to 64.2%	
			72	10µM reduced the proliferation to 42.2%	
				50 µM reduced the proliferation to 70.4%	
T24	Human bladder cancer	2.5 x 10^5 cells/12 well plates	24	Dose and time dependent inhibitory effect	[16]
SVG	Human brain cells	5 x 10^5 cells/ 25 cm^2 flask	96	40µM decreased the viability 71-98%	[17]
U-373 MG				40µM decreased the viability 88-100%	
H460	Human lung nonsmall carcinoma	1 x 10^5 cells/12 well plates	48	Dose and time dependent inhibitory effect IC_{50} = 40µM	[18]

TABLE II. CELL VIABILITY ASSAY USING PI STAINING

Type of Cell Line	Source of Cell Line	Cell Density	Hours of Treatment (Hours)	Effect of AE	Authors
SCC-4	Human tongue cancer cells	2 x 10^5 cells/ 12 well plates	24	*LC_{50} = 48.53±1.12 µM	[19]
NPC-TW 039	Human nasopharyngeal carcinoma cells	3 x 10^4 cells/ 24 well plates	36	60µM reduced cell viability at 49.36±4.2%	[20]
NPC-TW 076				60µM reduced cell viability at 43.36±2.49%	

C. Light-Absorbency Assay Systems

Indirect methods primarily provide data that reflect consistently the number of living cells. The procedures involved should theoretically unaffected with intrinsic or extrinsic factors. Among the indirect methods are Sulforhodamine B assay that measures the amount of protein [21-22] and other evaluating the synthesis of nucleic acid [23]. More common indirect method that is commercially available nowadays is the use of tetrazolium salts. It is a colorimetric assay which is based upon the bio-reduction of tetrazolium salts to a strongly colored formazan dye. The activity of the mitochondria dehydrogenase inside the culture is responsible to cleave the tetrazolium salt causing the increased formation of formazan that directly reflect the number of living cells. These systems are performed based on light absorbency using ELISA reader [24]. Among the assay that shared a similar principle are MTT [25], XTT [26] and MTS [27].

In this paper, we have presented some of the literatures related to our study that exploits the use of tetrazolium salts, investigating the anti-cancer or cytotoxicity activity of the AE in different type of cell lines (Table III and Table IV).

TABLE III. CELL VIABILITY ASSAY USING MTT TEST

Type of Cell Line	Source of Cell Line	Cell Density	Hours of Treatment (Hours)	Effect of AE/IC_{50} Value
B16	Murine melanoma cells	1 x 10^4 cells/96 well plates	24	Dose dependent inhibitory effect [28]
A375	Human melanoma cells			
B16-F10	Murine melanoma cells	-	48	IC_{50} = 60µM [29]
HeLa	Human cervical cancer cells	1 x 10^4 cells/96 well plates	24, 48, 72, 96, 120	Dose and time dependent inhibitory effect [30]
MGC-803	Human gastric cancer	1 x 10^4 cells/96 well plates	24, 48, 72, 96, 120	Dose and time dependent inhibitory effect [31]
C6	Rat glioma cells	3 x 10^4 cells/96 well plates	24	Dose and time dependent inhibitory effect [32]
HL60	Human leukemia cells	1 x 10^4 cells/96 well plates	12, 24, 48, 72	Dose and time dependent inhibitory effect [33]

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TABLE IV. CELL VIABILITY ASSAY USING XTT TEST

Type of Cell Line	Source of Cell Line	Cell Density	Hours of Treatment (Hours)	IC_{50} Value
AGS [34]	Human gastric carcinoma cells	-	48	-
			72	Below 0.07 mM
NCI-N87 [34]			48	-
			72	Between 0.15 and 019 mM
Hep G2 [35]	Human hepatoma cells	1×10^4 cells/96 well plates	48	11.77 ± 0.02 µg/ml
Hep 3B [35]				15.67 ± 0.01 µg/ml

III. METHODOLOGY

A. Cell Line

Estrogen-positive breast cancer cells, MCF-7 (American Type Cell Collection), were cultured as monolayer in complete RPMI 1640 media (GIBCO, Invitrogen USA), supplemented with 10% of fetal bovine serum (GIBCO Invitrogen, USA) and 1% of antibiotics 10 000 IU penicillin and 10000 ug/ml streptomycin (CELLGRO Mediatect, USA). The cells were maintained in humidified atmosphere of 5% CO_2, at 37°C in T25 flask (Orange Scientific, Belgium). For downstream application, exponentially growing stage of the cells was used throughout the assays.

B. Aloe Emodin Treatment

Aloe-emodin (Sigma Chemical, St. Louis, MO, USA) stock solution was dissolved in DMSO (Sigma Chemical) at 500µM. The final concentration of DMSO in the cell culture media was <0.1%.

C. WST-1 Assay

MCF-7 cells were seeded in 96-well microplates with seeding density of 40×10^3 cell per well as a monolayer in complete 100µL culture media. After 24 hours, the media were replaced with 100µL new prepared freshly media consisting with AE at different treatment concentrations (0, 10µM, 25µM, 50µM 75µM, 100µM, 125µM and 150µM). AE was prepared using DMSO as diluents. Cells cultured in complete media with 0.01% DMSO was used as control.

TAM was used as positive control. All treated cells were then incubated at different time of treatment (24 hours, 48 hours and 72 hours) in humidified atmosphere 37°C and 5% CO_2. Immediately after treatments, 10µL of 4-[3- (4-iodophenyl) -2- (4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate, (WST-1), monosodium salt was added into each well and were incubated for another 90 minutes. Microplates were shaken for at least 1 minute and the measurement of absorbance of the treated samples against a background control was carried out using Elisa Reader (Perkin Elmer, Maltham, USA) with photometric detection at 450 nm.

D. Statistical Analysis

One-Way ANOVA analysis was performed using SPSS software version 16.0 to compare groups at different concentrations of AE. All presented data values were expressed in mean ± S.E.M and significance was set at $p<0.05$. Each treatment was conducted in triplicates and repeated for at least three times.

IV. RESULTS AND DISCUSSION

A few staining techniques can be conducted to examine the viability of cells. Indirect method offers more options as some of the procedures could tolerate with a larger number of samples. The appropriate methods rely more on the facilities available and the nature of the samples itself. In routine staining procedure, certain particular samples encounter difficulties in producing one single cell suspension [6]. Moreover, misinterpretation to discriminate the dead and viable cell makes this technique cumbersome [36-37]. Time also is critical as blue dye used in trypan blue exclusion test increases over the time from the onset of addition [38]. Time consuming is another disadvantage for a larger number of samples that used counting method even though the existing of the electronic counter [11, 38].

Due to relatively insensitive and inconvenient setting of staining procedure, we determined to choose WST-1 assay as to consider the availability of our instrument. Besides no additional solubilisation step as in the MTT assay [39], the reagent is also more stable and sensitive compared to MTT, MTS or XTT assays [40-42]. Our present findings using WST-1 assay could provide a new knowledge and good comparison in term of different techniques and cell lines used for the assessment of anti-cancer activity in AE.

In this paper, we have presented our results on the capacity of AE to inhibit the proliferation of breast cancer cells, MCF-7. As compared to the standard positive control, TAM, AE has efficiently inhibited the viability of MCF-7 in both time and dose dependant manner. Using One-Way ANOVA with p-value less than 0.05, AE has significantly limiting the viability MCF-7 cells with IC_{50}<130µM after 24 and 48 (Fig. 1 and Fig. 2) hours of treatment. The IC_{50} has tremendously reduced to 80µM after 72 hours of treatment (Fig. 3). The results of the IC_{50} at different time of treatments are simplified in Table V.

Concordance with most of the literatures [43], our preliminary results have verified the potential of AE as a chemo-preventive agent in cancer prevention especially in breast cancer management. To our knowledge, no study has been done to investigate the effect of AE on estrogen-positive breast cancer cells, MCF-7. Our preliminary result could be a strong foundation to extend the study by looking depth in to the underlying molecular mechanism of the anti-cancer activity of AE. Future work at translational level should be considered as well as to expand the study at *in vivo* setting.

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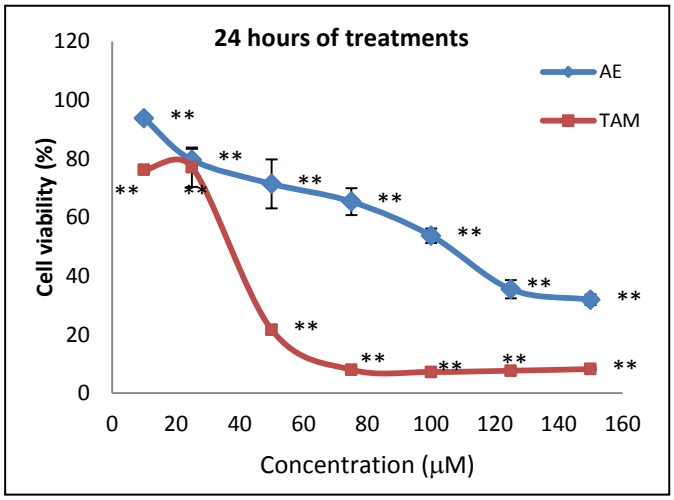

Fig. 1. Effects of aloe emodin (AE) and tamoxifen (TAM) on the viability of MCF-7 cells after 24 hours of treatments.

As shown in Fig. 1, Aloe emodin (AE) inhibited the viability of MCF-7 cells by 6% to 68% ($p<0.05$, $n=3$) beginning at 10μM to 150μM with IC_{50} value of 104μM ($p<0.05$, $n=3$) after 24 hours of treatment. While, tamoxifen (TAM) inhibited the viability of MCF-7 cells by 24% to 92% ($p<0.05$, $n=3$) beginning at 10μM to 150μM with IC_{50} value of 36μM ($p<0.05$ $n=3$). The data presented as mean ± S.E.M and was significant as compared to control, $p<0.05$. ** = significant $p<0.05$.

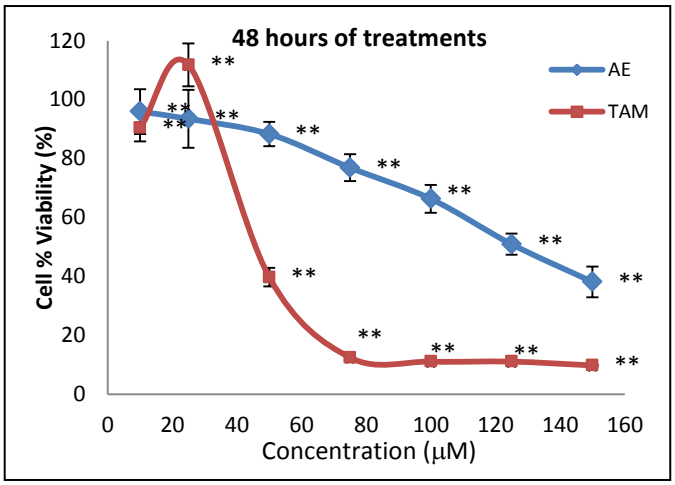

Fig. 2. Effects of aloe emodin (AE) and tamoxifen (TAM) on the viability of MCF-7 cells after 48 hours of treatments.

In Fig. 2, Aloe emodin (AE) inhibited the viability of MCF-7 cells by 4% to 62% ($p<0.05$, $n=3$) beginning at 10μM to 150μM with IC_{50} value of 125μM ($p<0.05$, $n=3$) after 48 hours of treatment. While, tamoxifen (TAM) inhibited the viability of MCF-7 cells by 10% to 90% ($p<0.05$, $n=3$) beginning at 10μM to 150μM with IC_{50} value of 46μM ($p<0.05$ $n=3$). The data presented as mean ± S.E.M and was significant as compared to control, $p<0.05$. ** = significant $p<0.05$.

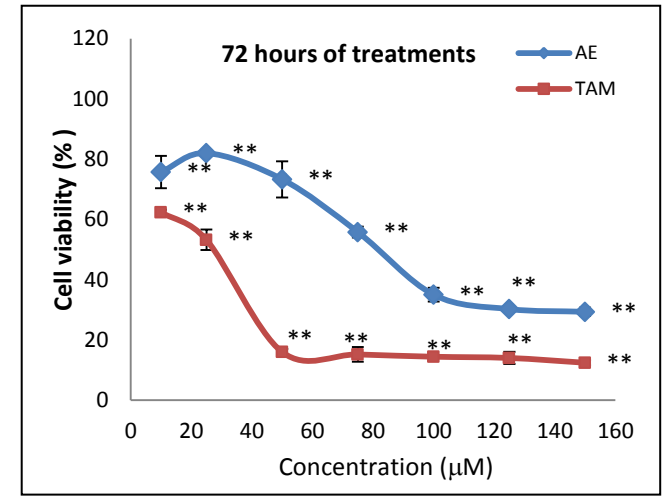

Fig. 3. Effects of aloe emodin (AE) and tamoxifen (TAM) on the viability of MCF-7 cells after 72 hours of treatments.

As stated in Fig. 3, Aloe emodin (AE) inhibited the viability of MCF-7 cells by 24% to 71% ($p<0.05$, $n=3$) beginning at 10μM to 150μM with IC_{50} value of 80μM ($p<0.05$, $n=3$) after 72 hours of treatment. While, tamoxifen (TAM) inhibited the viability of MCF-7 cells by 38% to 88% ($p<0.05$, $n=3$) beginning at 10μM to 150μM with IC_{50} value of 27μM ($p<0.05$ $n=3$). The data presented as mean ± S.E.M was significant as compared to control, $p<0.05$. ** = significant $p<0.05$.

TABLE V. RESULTS OF THE IC_{50} AT DIFFERENT TIME OF TREATMENTS

Treatments	Hours of Treatments (Hours)		
	24	48	72
Aloe emodin (μM)	104	125	80
Tamoxifen (μM)	36	46	27

Table V represent the IC_{50} of AE and TAM at different time of treatments (24, 48 and 72 hours). The effect of aloe emodin on MCF-7 cell was significantly seen after 72 hours treatment with the IC_{50} reduced to 80μM.

V. CONCLUSION

As conclusion, AE has a potential to inhibit the viability of estrogen-positive breast cancer cells, MCF-7 in both time and dose dependent manner. The limiting viability capability of AE in MCF-7 was observed after 24, 48 and 72 hours of treatment. The IC_{50} of AE has markedly been reduced to 80μM after 72 hours treatment. These findings have verified the potential of AE to be a chemo-preventive agent in management of breast cancer patients. Additionally, the data obtained could provide a good comparison with the available related research as the different technique and cell line were used throughout the experiment. The next step to be undertaken is to extend the study by looking on the gene expression analysis as well as to investigate the regulation involve at protein level. *In vivo* studies could be considered to widen the possibility for future prospect of clinical setting experiment.

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