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Induction of apoptosis by chalepin through phosphatidylserine externalisations and DNA fragmentation in breast cancer cells (MCF7)

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ABSTRACT

Aims: Chalepin, a naturally occurring compound isolated from *Ruta angustifolia* have been shown to exert a promising anticancer activity through various mechanisms. Hence, the need to investigate the apoptotic inducing ability of chalepin in MCF7 cells by various detection assays.
Materials and methods: Cytotoxicity screening of chalepin against MCF7 cells was conducted using SRB assay. Apoptosis induction was examined by established morphological and biochemical assays including phase contrast and Hoechst/PI staining fluorescence microscope. Similarly, Annexin-V/FITC and TUNEL assays were conducted using flow cytometry whereas caspase-3 activity was evaluated using microplate reader.
Key findings: The result indicates remarkable cytotoxic activity against MCF7 cells, whereas it shows moderate cytotoxic activity against MDA-MB231 cells. Interestingly, chalepin did not present any toxicity against MRC5 normal cell line. Morphological examination using both phase contrast and fluorescence microscope displays typical apoptotic features such as membrane blebbing, DNA fragmentation, chromatin condensation and apoptotic bodies' formation following chalepin treatment against MCF7 cells at different concentration for 48 h. Apoptosis induction is significantly associated with externalisation of phosphatidylserine, and DNA fragmentation in MCF7 cells chalepin treated cells when compared with control. The protein expressions of caspase-8, 9 and cleaved PARP1 were upregulated which correlated well with increased caspase-3 activity.
Significance: From our recent findings, chalepin was able to induced apoptosis in MCF7 cells and therefore, could be evaluated further as a potential source of anticancer agent for cancer treatment such as breast cancer.

1. Introduction

Cancer continues to be a devastating disease and among the leading causes of death all over the world [1–4]. Specifically, breast cancer has been identified as one of the primary cancer causes of death in women. Several factors such as age, mutations in BRCA1/2 genes, less frequent p53 gene and endocrine disruptors are associated with increased risk of breast cancer development and its subsequent diagnosis, prevention and treatment [5–7].

Currently, a concerted effort is being made towards searching, developing and promoting anticancer drugs for cancer prevention and or/treatments through various approaches. As such, a number of drugs have been approved including trastuzumab, vorinostat, imatinib, bortezomib [8]. Recently, Liu et al. [9], reported the application of aptamers for breast cancer diagnosis and therapy. Other methods are also currently being studied [10,11]. The inhibitory activity of iron chelators and lipoic acid on MCF7 breast cancer cells as an anticancer strategy has also been reported [12,13]. Our study on the other hand focused on a small molecule "chalepin" isolated from *Ruta angustifolia* which have been reported to show promising anticancer activity in A549, via apoptosis induction and signalling pathways [14,15].

Apoptosis is a mechanism by which cell commit suicide and get eliminated from the system. Apoptosis induction has been one of the primary strategies by which chemotherapeutic agents exert their anticancer activities [16,17]. Various features including morphological changes characterise it. These include loss of mitochondrial membrane potential, activation of caspases and DNA fragmentation; which has been found to proceed by both extrinsic and intrinsic pathways [16–18]. These two pathways are reported to have been triggered by several factors and signalling cascades which can be initiated by different chemotherapeutic agents [3,16,17].

Apoptosis is primarily an important cell toxicity pathway which can be used to investigate new antitumor agents. Apoptosis, unlike necrosis, proceeds via a series of distinct morphological and biochemical steps

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that occur in the nucleus, mitochondria as well as the cytoplasm of the dying cells. One of the biochemical features, caspase proteolytic activity, has been identified as a hallmark of apoptosis [19–21]. Moreover, sensitising cancer cells to drug-induced apoptosis is a vital strategy to overcome carcinogenesis. Therefore, more research is now geared towards novel pro-apoptotic chemotherapeutic drug agents from plant sources in the search of inhibitors for cancer. Hence, the investigation of chalepin as an apoptosis-inducing compound will further contribute to this endeavor.

2. Material and methods

2.1. Cell lines, chemicals and biochemical

MCF7 cells, human hormone-dependent breast cancer, MDA-MB231 cells, human non-hormone-dependent breast cancer, and MRC5, human normal lung fibroblast cells were purchased from America Tissue Tyce Collection (ATCC), Annexin-V/FITC assay kit and caspase-3 assay kit were purchased from BD Bioscience whereas APO-BrdU TUNEL assay kit was obtained from life technologies. Sulforhodamine B (SRB), Hoechst 33342 dye, Propidium Iodide (PI) and Fetal Bovine Serum (FBS) were obtained from Sigma-Aldrich. Dulbecco's Modified Eagles Medium (DMEM), Eagle's Minimum Essential Medium (EMEM) Accutase, penicillin/streptomycin (P/S), amphotericin B (AmpB), sodium pyruvate, Non-Essential Amino Acids (NEAAs). RIPA lysis buffer and Phosphate Buffer Saline (PBS) were obtained from Nacalai Tesques, Inc. Primary (mouse and rabbit; 1 mg/ml) and secondary (anti-mouse and anti-rabbit; 1.5 mg/ml) antibodies were obtained from Thermo Scientific. All other chemicals and reagents were standard and for analytical grades.

2.2. Plant material

Ruta angustifolia whole plant was purchased at a Nursery in Sungai Buloh, Selangor, Malaysia; on 15/09/2015. The plant sample was identified at the Institute of Biological Sciences, Faculty of Science, University of Malaya; by a Botanist; Dr Sugamaran Manickam. A voucher specimen (reference no. KLU48128) was deposited at Herbarium of Rimba Ilmu, University Malaya, Kuala Lumpur, Malaysia.

2.3. Extraction and isolation of extract and pure compound

The aerial part of *Ruta angustifolia* was extracted by adopting a method as reported by Richardson et al. [15] and Suhaimi et al. [22]. Similarly, the chalepin compound was isolated from the chloroform extract using high-performance liquid chromatography (HPLC). The compound structure was determined by gas chromatography–mass spectrometry (GCMS) and nuclear magnetic resonance spectroscopy (NMR) as described by Richardson et al. [15] and Suhaimi et al. [22]. For use in downstream experiments, chalepin was dissolved in dimethyl sulfoxide (DMSO) for treatment purpose.

2.4. Cell culture

MCF7 and MDA-MB-231 cells were cultured in DMEM while MRC-5 cells were cultured in EMEM. All media used were augmented with 10% v/v FBS, 2% v/v penicillin/streptomycin, and 1% v/v amphotericin B.

2.5. In vitro cytotoxicity screening

Cells at a density of 50,000/ml for MCF7, MDA-MB-231, and MRC-5 cells were seeded onto sterile 96-well plates. The plated cells were incubated for 24 h allowing cells adherence. Media was removed and substituted with a new one containing chalepin of different concentrations at 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 μg/ml. The plates were then incubated at 37 °C, 5% CO_2 for periods of 24, 48 and 72 h. Plain media with 0.5% of DMSO was used for the cells serving as a negative control instead. The SRB assay was done adopting a method reported by Phang et al. [23]. A microplate reader (Biotek Synergy H1 Hybrid) was used to record the absorbance at 492 nm and 620 nm. Percentage inhibition, as well as cell viability of each sample, was calculated using the following expressions.

$$\%\text{inhibition} = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{sample}}{\text{Absorbance}_{control}} \times 100$$

The concentration of test agent that causes 50% inhibition or cell death (IC50 value) for each cell line was determined from dose-dependent response curves. The experiment was performed in triplicates.

2.6. Morphological analysis

MCF7 cells with a density of 3×10^5 cells/ml were plated into a culture plate and then incubated overnight. Treatment was done with chalepin using concentrations at 10, 20 and 30 μg/ml and further incubated at 37 °C and 5% CO_2 for 48 h. Untreated cells served as a control. Sample preparations were done as earlier described [15,22]. Alterations in the cellular and nuclear morphology of the cells were observed at 40× magnification using phase contrast microscope, Zeiss Axio Vert. A1, and Leica fluorescence microscope, DM6000B respectively.

2.7. Apoptosis detection using Annexin-V/FITC assay

MCF7 cells with a density of 5×10^5 cells/ml were plated into a culture plate and then incubated overnight. Treatment was done with chalepin using concentrations at 10, 20 and 30 μg/ml and further incubated at 37 °C and 5% CO_2 for 48 h. Untreated cells serve as a negative control, while positive control cells were treated with doxorubicin. After the incubation periods, cells were harvested and washed with cold PBS. The cells were then resuspended with Annexin V binding buffer, doubled stained using Annexin V/FITC and PI solutions; and incubated at room temperature in the dark for 15 min. BD FACS CANTO II flow cytometer was used for apoptosis detection. Quadrant statistics was used to analyse cell population distribution in different quadrants. Viable cells were represented in the lower left quadrant, early apoptotic cells in the lower right quadrant, late apoptotic/secondary necrotic cells in the upper right quadrant represents, and dead cells were represented in the upper left quadrant.

2.8. Assessment of caspase-3 activity

MCF7 cells with a density of 5×10^5 cells/ml were plated into culture plate and then incubated overnight. Treatments were done with chalepin using concentrations of 10, 20 and 30 μg/ml and further incubated at 37 °C, 5%, and CO_2 for 48 h. Untreated cells serve as a negative control, while positive control cells were treated with doxorubicin. After the incubation periods, the cells were harvested and washed using cold PBS. The samples were resuspended using cell lysis buffer and incubated on ice for 30 min. The experiment was conducted following the procedure described in the manufacturer's instructions manual. The amount of aminomethylcoumarin (AMC) fluorescent was measured at an excitation wavelength of 380 nm with an emission wavelength range of 420–460 nm using a microplate reader, BioTek, Synergy H1 Hybrid. Consequently, apoptotic cell lysates with activated caspase-3 yield a significant emission as related to non-apoptotic cell lysates.

2.9. Detection of DNA fragmentation using TUNEL assay

MCF7 cells with a density of 1×10^6 cells/ml were plated into culture flask and incubated overnight. Treatments were done with chalepin using concentrations of 10, 20 and 30 μg/ml and further incubated at 37 °C, 5%, and CO_2 for 48 h. Untreated cells serve as a negative control, while positive control cells were treated with doxorubicin. After the incubation periods, the cells samples were then harvested, washed using cold PBS, fixed in 1% formaldehyde for 1 h on ice and later fixed in 70% ethanol overnight to permeabilize the cells. Following fixation; DNA labelling and staining was done by adopting the manufacturer's instructions. Percentage DNA fragmentation was analysed using BD FACS CANTO II flow cytometer.

2.10. Western blot analysis

MCF7 cells with a density of 2×10^6 cells/ml were seeded into a culture flask and incubated overnight. The cells were treated using chalepin at 20 μg/ml for 6, 12, 18, 24 and 48 h. After each treatment period, cells were harvested and washed using PBS. Proteins from the whole cell lysate were extracted adopting procedure described by Phang et al. [23]. Protein quantification, western blot procedures, and band quantification were conducted as described by Phang et al. [17].

2.11. Statistical analysis

All data were expressed as a mean ± standard deviation of three (3) replicates. Statistical significant comparison between the means was determined by one-way analysis of variance (ANOVA) and Turkey post-hoc test at 5% confidence limit ($P < 0.05$) using SPSS version 23.

3. Result

3.1. Isolation and cytotoxicity screening of chalepin

Chalepin was isolated from the chloroform fraction using HPLC with a retention time of 28.890 min. The purity of the isolated chalepin was according TLC analysis that shows single spot having same Rf value with the standard chalepin as well as GC–MS analysis which shows single chalepin peak from TIC and **314** molecular weight of chalepin from MS profile in conformity with the structure of the chalepin (Fig. 1). From our results, chalepin displayed remarkable cytotoxicity against MCF7 cells; with moderate activity against MDA-MB-231 cells. Interestingly, the compound did not exert any toxicity against MRC5 normal cell used (Table 1).

3.2. Effect of chalepin on MCF7 cells morphology

Following treatment for 48 h at different concentrations, chalepin significantly affects both cellular and nuclear morphology (Fig. 2a & b). From the results, chalepin drastically induced morphological changes on MCF7 cells in a dose-dependent manner leading to the consequent formation of typical apoptotic features including cell shrinkage, membrane blebbing and apoptotic bodies. Similarly, chalepin induced nuclear morphological changes resulting in concomitant cell number reductions, the existence of cells with condensed chromatin and dead cells with fragmented DNA.

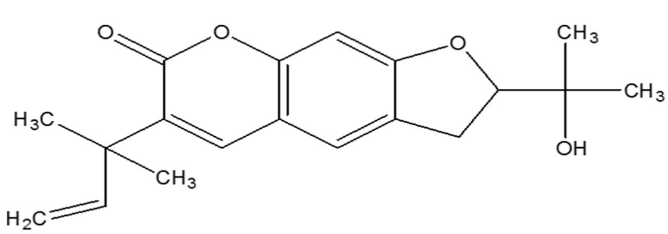

Fig. 1. Structure of chalepin (MW314).

Table 1
Cytotoxicity activity of chalepin against MCF7, MDA-MB231, and MRC5 cells.

Cell lines	Time of incubation (h)	IC_{50} (μg/ml)	
		Chalepin	DOX (72 h)
MCF7	24	36.0 ± 3.3	
	48	21.8 ± 1.9	
	72	8.5 ± 1.4	0.10 ± 0.00
MDA-MB-231	24	95.8 ± 1.3	
	48	48.9 ± 1.0	
	72	19.8 ± 0.8	0.07 ± 0.01
MRC-5	72	> 100	0.35 ± 0.01

Values are expressed as mean ± SD of 3 replicate from 3 experiments.

3.3. Increased phosphatidylserine externalisation in chalepin treated MCF7 cells

Following treatment for 48 h at different concentrations, chalepin significantly ($P < 0.05$) increased the number of Annexin-V/FITC positive cells in MCF7 cells compared to the untreated cells (Fig. 3). The result indicated a dose-dependent increase of early and late apoptotic cells with chalepin treatment. It also increased the number of Annexin-V/FITC positive cells, thus confirming the apoptotic inducing ability of chalepin in MCF7 cells.

3.4. Chalepin increased caspase-3 activity in MCF7 cells

Following treatment for 48 h at different concentrations, chalepin significantly ($P < 0.05$) increased caspase-3 activity in MCF7 cells as compared to untreated cells in a dose-dependent manner (Fig. 4). Similarly, cleaved caspase-8 and caspase-9 expression were upregulated, resulting in consequent cleavage of PARP1 to cleaved-PARP1.

3.5. Chalepin increased DNA fragmentation in MCF7 cells

Following treatment for 48 h at different concentrations, chalepin significantly ($P < 0.05$) increased DNA fragmentation in MCF7 cells when compared with the untreated cells (Fig. 5).

3.6. Effect of chalepin on some pro- and anti-apoptotic proteins in MCF7 cells

In this study chalepin treatment in MCF7 cells was found to downregulate the antiapoptotic Bcl2 protein, whereas proapoptotic Bid was upregulated. Similarly, Bax expression decreased in the cytosol (Fig. 6a). On the other hand, phosphorylated P38, as well as P53 suppressor proteins were also found to be upregulated following chalepin treatment in MCF7 cells (Fig. 6b).

4. Discussions

Though there are available drugs used for prevention and treatment, many of the drugs are toxic and costly. Apart from that, some of the drugs become ineffective as a result of resistance developed by cancer cells [24,25]. It is well-known that most chemotherapeutic drugs are synthesised to recognise and target a specific protein or gene associated with cancer proliferation or survival pathway [24]. The specificity of the chemotherapeutic drugs could also be a reason why the drugs are ineffective since multiple signalling pathways are channelled to oncogenic events by altering normal cellular homeostasis during tumor initiation and progression [3].

Apoptosis is an essential mechanism by which damage and old cells die, and cellular debris are eliminated from the biological system without affecting other tissues. However, cancer cells resist cell death since they avoid apoptosis and continue to proliferate. Therefore,

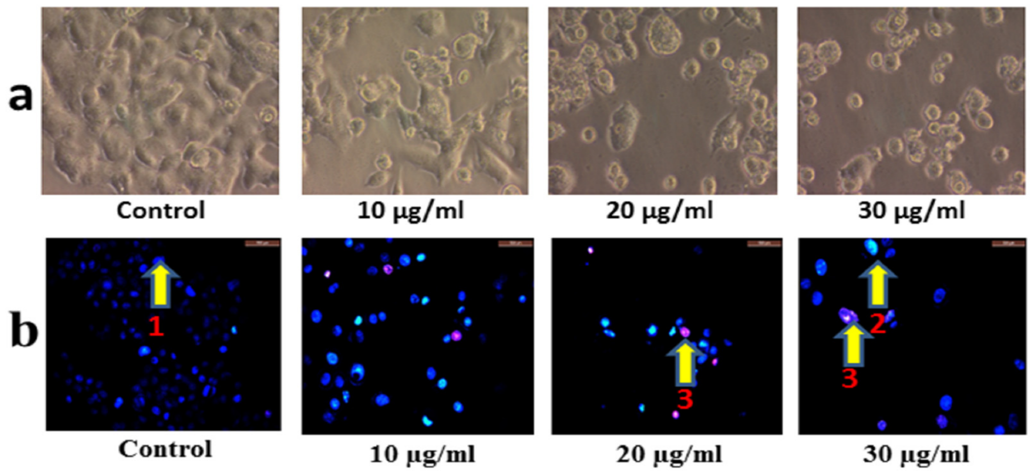

Fig. 2. Effect of chalepin treatment on cellular and nuclear morphology. (a) Cellular morphological changes observed under phase contrast microscope. (b) Nuclear morphological changes following Hoechst/PI staining and observed using fluorescence microscope; after 48 h of incubation. Viable cells (1), cells with condensed chromatin (2) and dead cells with fragmented DNA (3) were observed in MCF7 cells.

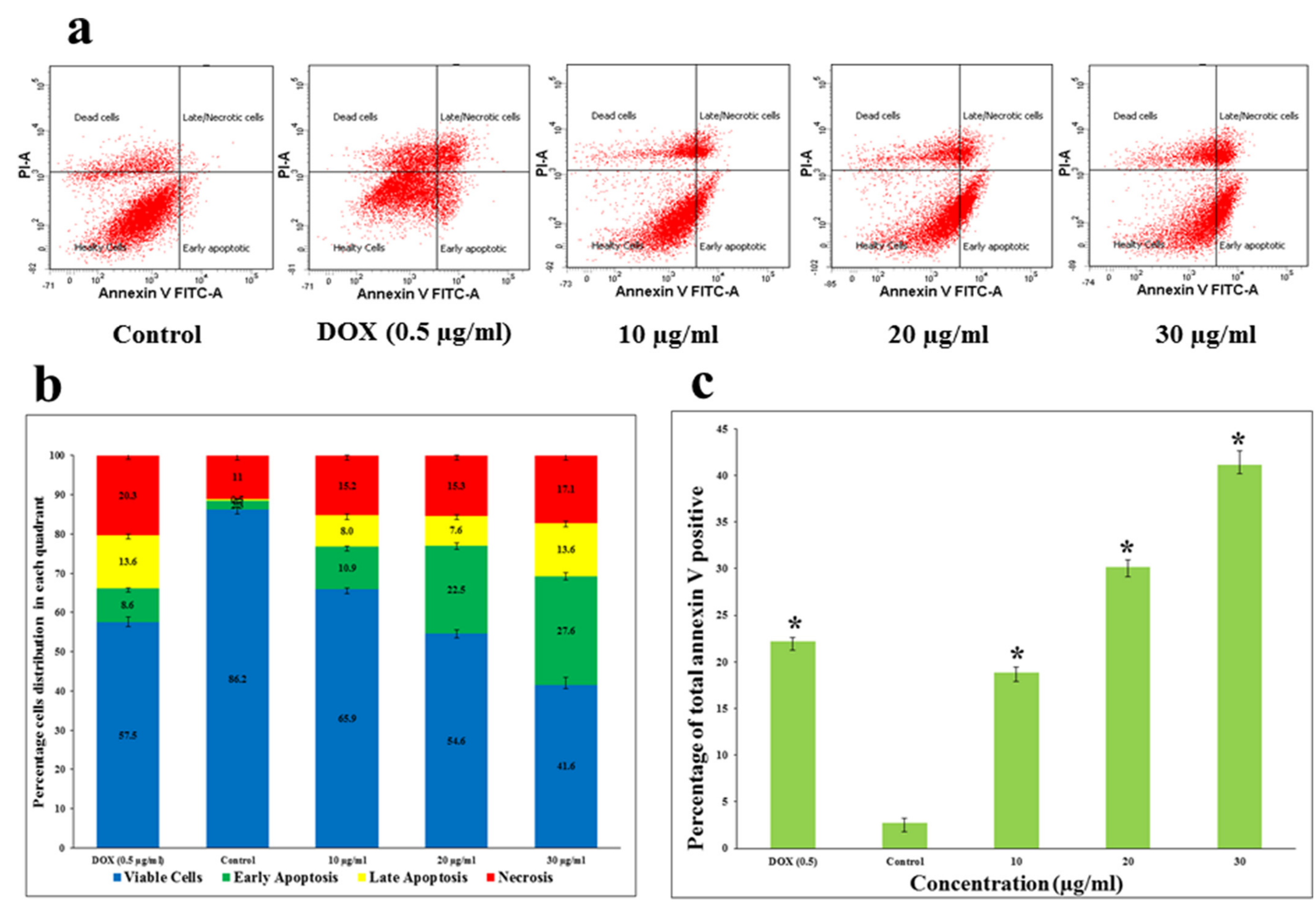

Fig. 3. Effect of chalepin treatment on externalisation of phosphatidylserine. (a) Annexin V density plot. (b) Percentage cell distribution in each quadrant. (c) Percentage total Annexin V positive cells; in MCF7 cells following chalepin treatment at different concentrations for 48 h. Data are expressed as mean ± SD (n = 3). *P < 0.05 vs control group.

apoptosis manipulation using chemotherapy could be an essential strategy to treat cancer and any compound with the ability to overcome apoptosis resistance by cancer cells may have potential to be used for cancer treatment. Moreover, developing a new therapeutic product requires an evaluation of the in vitro cytotoxicity of the compound as an essential step. Consequently, our study suggested that chalepin treatment resulted in strong cytotoxicity against MCF7 cells which may stimulate cell death via apoptosis. However, doxorubicin which is an established drug used for cancer treatment affect both cancer and the healthy cells, indicating that chalepin could be explored as a better complementary therapy for cancer treatments as it causes no observable toxicity against healthy cells. It has been reported earlier that most therapeutic drugs used nowadays for cancer treatment also cause damage to healthy cells [26]. Our study is consistent with the report of Richardson et al. [15]; who reported remarkable cytotoxicity of chalepin against A549. Recently, Suhaimi et al. [22], reported cytotoxicity of rutamarin, a similar compound to chalepin also isolated *Ruta angustifolia*.

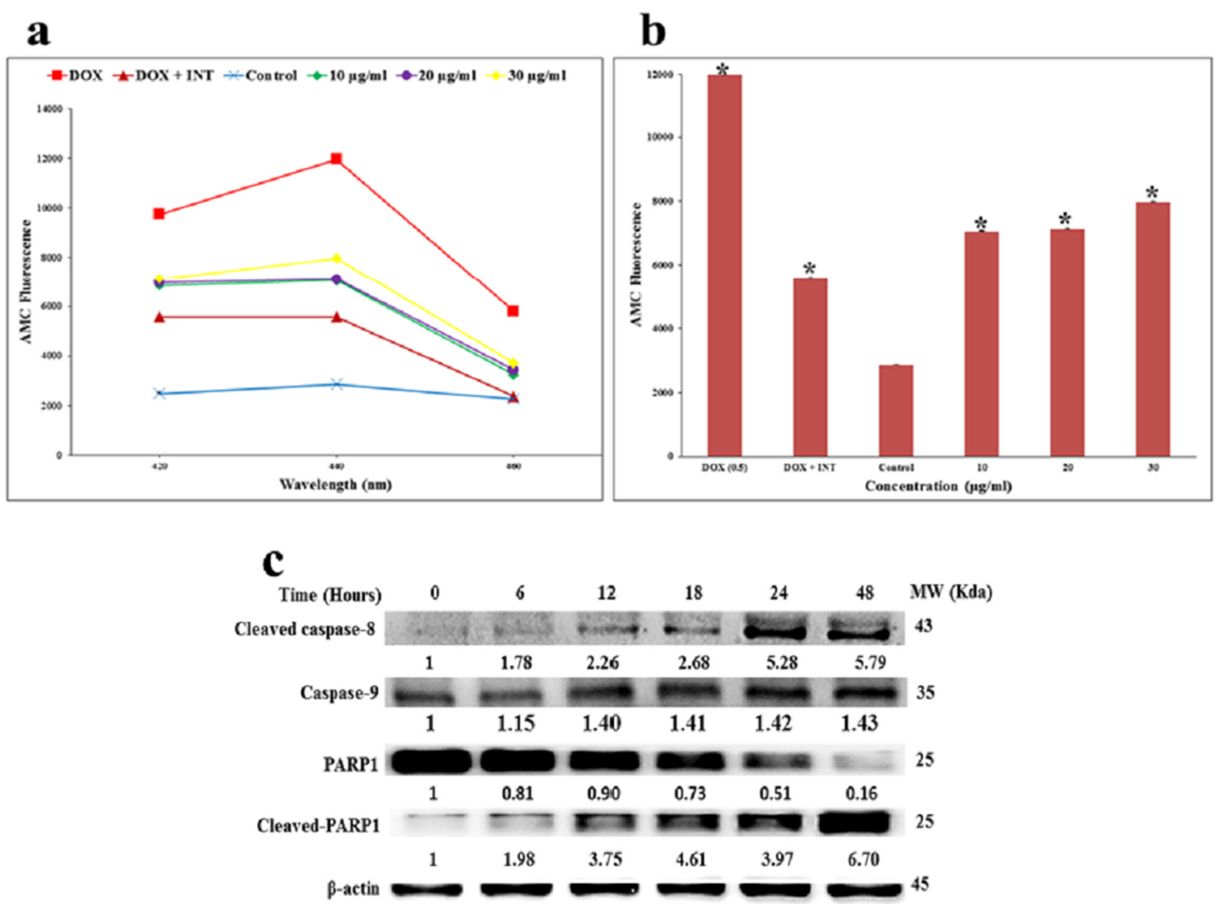

Fig. 4. Effect of chalepin treatment on caspase-3 activity. (a) Caspase-3 substrate-fluorescence count. (b) Increased caspase-3 activity; in MCF7 cells following chalepin treatment at different concentrations 48 h. Data were measured at excitation of 380 and emission of 420, 440 and 460 nm and expressed as mean ± SD (n = 3). *P < 0.05 vs control group. (c) Effect of chalepin treatment in MCF7 cells on some apoptotic proteins.

Apoptotic cells typically show distinct morphological changes including membrane blebbing, nuclear fragmentation, chromatin condensation with associated margination of chromatin to the nuclear membrane, and apoptotic bodies formation [27]. Our study, therefore, suggested that chalepin treatment promotes apoptosis in MCF7 cells as observed using both phase contrast and fluorescence microscopy (Fig. 2). After 48 h of different concentrations of chalepin incubation; typical apoptotic features such as reduced number of cells, cell shrinkage, vacuolisation, chromatin condensation, cytoplasmic and nuclear fragmentation; and finally, apoptotic bodies' formation was all observed in MCF7 cells.

During apoptosis, phosphatidylserine located in the inner leaflet is translocated to the outer leaflet of the plasma membrane thus exposing the apoptotic cells to macrophages attack to initiate early recognition and quick phagocytosis by the neighbouring cells with limited effect to the surrounding tissues [15]. This phenomenon can be detected using Annexin-FITC/PI double staining and flow cytometry to determine early apoptosis and distinguished between apoptotic and dead cells. The same technique has earlier been used to discriminate between apoptosis and necrosis as reported by Sawai and Domae [28]. Our present study, therefore, suggested that chalepin induced externalisation of phosphatidylserine in MCF7 chalepin-treated thereby increasing both early and late apoptosis which was detected using Annexin-FITC/PI and flow cytometry. It has been observed that Annexin-V positive cells increased correlates to the early and late apoptosis increases with increased concentration of chalepin in MCF7 cells. Furthermore, exposure of phosphatidylserine on the outer leaflet results in mitochondrial membrane potential alterations which causes release of intermembrane space proteins, caspases activations, and translocation of caspase-activated DNase leading to cleavage of important substrate such as DNA and PARP1 as some of the biochemical features that characterised apoptosis [27]. Similar observations of increased early and late apoptotic cells based on time- and dose-dependent have been reported [15,22].

Apoptosis mechanism is typically associated with activation of an aspartame-specific class of proteases known as caspases which are expressed as inactive zymogens and becomes activated upon cleavage by other caspases via caspase activation cascades [29]. They are classified as initiators (caspase-2, -8, -9 and -10) and executioners (caspase-3, -6 and -7) caspases that involved in cell death. Among these, caspase-3 is the most crucial in apoptosis induction as it involved for both extrinsic and intrinsic pathways as well as cleavage of the majority of substrates related to apoptosis [29–31]. Although, it has earlier been reported that MCF7 cells do not express caspase-3 due to deletion of CASP-3 gene [29,32]. However, other executioners such as caspase-6 or -7 may be involved in the increases of caspase-3-like activity of chalepin in MCF7 cells (Fig. 4). The observed increased fluorescence emission in our study may be due activation of other caspase cascades which results to continues cleavage of the caspase-3 substrate due to apoptosis in MCF7 cells after chalepin treatment. The activation was also confirmed by increased expression of cleaved-caspase-8, caspase-9, and cleaved-PARP through western blot analysis (Fig. 4c). Our result suggests that chalepin might have induced caspase-8 and caspase-9 activation which consequently activates caspase-3 resulting in continues cleavage of PARP to cleaved-PARP. Subsequently, cleaved-PARP initiated irreversible apoptosis in MCF7-chalepin treated as compared to untreated. Caspases including caspase-3, 8 and 9 play a vital role in apoptosis initiation and execution. Caspases have been reported to be either

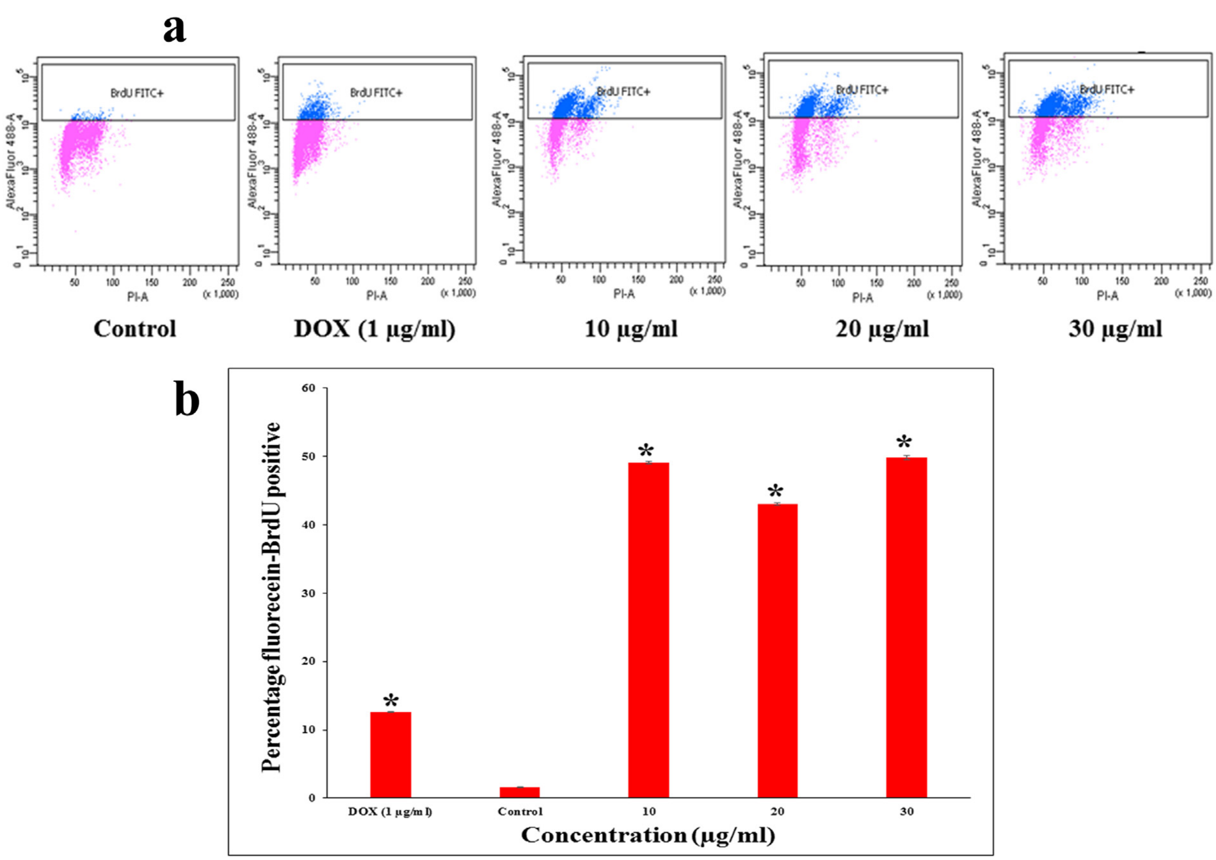

Fig. 5. Effect of chalepin treatment on DNA fragmentation. (a) TUNEL assay dot plots. (b) Percentage DNA fragmentation. Data were presented as mean ± SD (n = 3). *P < 0.05 vs control group.

initiator such as caspase-8 and 9 or executors such as caspase-3 of apoptosis. Thus their activation results in both extrinsic and intrinsic apoptotic pathways. This result agreed with the published reports [15,17,22]. The result may also suggest that chalepin induces mitochondrial membrane potential alterations causing the release of cytochrome *c* which binds to apoptotic proteases activating factor 1 (Apaf1) which activates caspase-3 and subsequently triggered induction of intrinsic apoptotic pathway. Accordingly, chalepin-induced apoptosis facilitated by caspase-3 activation and could be associated with activation of both extrinsic and intrinsic apoptotic pathways due to caspase-8 and caspase-9 expressions respectively. Importantly, the increased caspase-3 activity causes proteolysis of different substrates including PARP1 which may also induce some typical morphological and biochemical changes associated with apoptosis observed in MCF7 cells following chalepin treatment.

It has shown that the DNA-laddering pattern in MCF7-chalepin treated corresponds to the amount of DNA fragmented as observed using flow cytometer. This result suggests that chalepin was able to induce stress in MCF7 cells resulting in DNA damage and fragmentation which is one of the typical characteristics of apoptotic cells. However, there were little or no apoptotic cells with fragmented DNA observed in the untreated cells; in contrast, a similar pattern of DNA fragmentation was observed in doxorubicin (DOX)-treated cells (Fig. 5). Apoptosis in cells associated with one of its biochemical hallmarks known as DNA degradation. In this case, endonucleases cleave DNA into double-stranded oligonucleosomal DNA fragments with sizes of about 180–200 bp [22,33,34]. Our result, therefore, further supported the mechanisms of apoptotic-inducing ability of chalepin in MCF7 cells which also commensurate with both increased Annexin-V positive cells, as well as morphological changes observed.

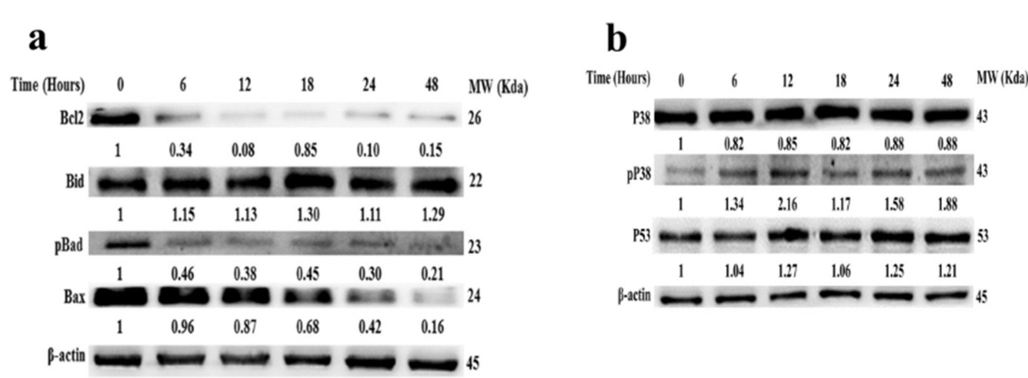

Fig. 6. Effect of chalepin treatment on some pro- and anti-apoptotic proteins. (a) Expression of Bcl2 family proteins. (b) Expression of P38 and P53 suppression proteins in MCF7 cells following chalepin treatment at different concentrations for 48 h.

Apoptosis in cells is firmly regulated by various pro- and anti-apoptotic family proteins. Thus, the dynamic expression of these proteins usually determines the fate of a cell to survive or die through apoptosis mechanism [35]. The increased expression of pro-apoptotic proteins such as Bax, pBad and Bid with the corresponding decreased in Bcl2 proteins (Fig. 6a), favored apoptosis induction due to chalepin treatment in MCF7 cells. Hence, the observed increased of pro-apoptotic proteins consequently stimulates cytochrome C released from the mitochondria thereby inducing apoptosis through intrinsic mechanism. Similar, observation was reported following neocarzinostatin treatment in MCF7 which causes condensation and fragmentation of cellular nuclei with consequence release of cytochrome C from the mitochondria into the cytosol [36].

The mitogen-activated protein kinase (MAPKs) family including P38 was reported to intercede several cellular behavioral responses to external stimuli. P38 has been implicated in various biological processes such as cell death, cell growth, differentiation, inflammation and apoptosis [37]. It has been reported that many anticancer compounds induced apoptosis by activating P38 MAPK signalling in various cancer types [38]. The activation of P38 in this study (Fig. 6a) may be due to stress following chalepin treatment in MCF7 cells which might confer its involvement in apoptosis induction. The activated P38 was found to initiate mitochondrial apoptosis pathway by enhancing transcription of pro-apoptotic genes and directly activating them. P38 activation thus, enhance activation of P53 tumor suppressor protein as well as translocation of the pro-apoptotic proteins Bax, pBad and Bid which then facilitate mitochondrial release of cytochrome C that induced apoptosis through activation of caspases with consequence cleavage of PARP resulting to apoptosis induction in the chalepin-treated MCF7 cells.

5. Conclusion

In summary, our study demonstrated the cytotoxicity of chalepin against MCF7 breast cancer cells and its apoptosis-inducing ability through both morphological and biochemical changes observed. Possible mechanisms for the apoptosis induction by chalepin in MCF7 cells include activation of P38 MAPK signalling pathway. This compound "chalepin" might, therefore, be considered as a potential candidate for development as an anticancer agent either as an adjuvant with the existing therapies or in combination with other current conventional anticancer drugs used for breast cancer treatment.

Conflict of interest statement

The authors declare that there no conflicts of interest.

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