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Dendrophthoe pentandra Induced Apoptosis and Cell Cycle Arrest at G1/S in Human Breast Adenocarcinoma Cells, MCF-7 via Up-Regulation of p53

Nik Aina Syazana Nik Zainuddin, Yusmazura Zakaria, Mohd Dasuki Sul'ain*

Biomedicine Programme, School of Health Sciences, Universiti Sains Malaysia, 16150 Kota Bharu, Kelantan, Malaysia.

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ABSTRACT

D. pentandra is a semi-parasitic plant with traditional claim of having antiproliferative and anticancer properties. However, the mechanism underlying anticancer activity of this plant remains unclear. This study aims to evaluate the antiproliferative activity of *D. pentandra* and its mechanism of action in cancer treatment. The extraction of *D. pentandra* was performed using methanol. The antiproliferative activity of *D. pentandra* methanol extract using MTT Assay showed inhibition on MCF-7 cell proliferation with IC_{50} of 10.65 ± 0.49 µg/ml. Hoechst 33258 nuclear staining displayed chromatin condensation, fragmented nuclei, and formation of apoptotic bodies upon treatment with IC_{50} of *D. pentandra* methanol extract. Mechanism of cell death in *D. pentandra* methanol extract-treated MCF-7 cells were performed by flow cytometric analysis. The analysis confirmed that *D. pentandra* methanol extract-treated MCF-7 arrested cell cycle distribution at the G1/S phase and induced apoptosis using AnnexinV/propidium iodide double staining. The mechanism of action was further confirmed by determination of protein involved in apoptosis pathway; Bcl-2, Bax, and p53. The findings suggested that the increased p53 was followed by an increase of pro-apoptotic Bax and decrease of anti-apoptotic Bcl-2 that led to apoptosis. In conclusion, the anticancer effect of *D. pentandra* methanol extract against MCF-7 cells by induction of apoptotic cell death is a promising approach for breast cancer treatment..

INTRODUCTION

Cancer is a major cause of death worldwide. Each year, about 37, 000 new cancer incidents and 22, 000 cancer deaths were reported in developing countries such as Malaysia (Fernandez, 2014). Among Malaysian women, one of three common cancer cases was breast cancer (Dahlui *et al.*, 2011). Hence, there is an urgent need to better control breast cancer case by developing new potent and economical approaches to combat the problem.

Surgery still remains as the most common option for breast cancer treatment with the radiotherapy and chemotherapy (Yaacob *et al.*, 2013; Yip *et al.*, 2014). However, the surgery involves expensive cost and tendency of cancers to invade other tissue by microscopic metastatic (National Breast Cancer Foundation, 2015).

Whereas, side effects of chemotherapy and radiation are limited by toxicity effects to healthy tissues in the body (Acton 2013; Yip *et al.*, 2014). Malaysian claimed that usage of herbal medicine is able to improve survivorship regardless of scanty evidence of their effectiveness (Soon *et al.*, 2009; Sooi and Keng, 2013). Hence, it is important to establish and initiate pre-clinical trials especially for the common local traditional herbal medicine.

In this study, *Dendrophthoe pentandra* (L.) Miq (*D. pentandra*) or known as mistletoe was chosen based on traditional claim and is easily available. *D. pentandra* is used in to treat diabetes, hypertension, cancer, skin infection and also for postpartum recovery in Indonesia (Ishizu *et al.*, 2002; Artanti *et al.*, 2012). Widowati *et al.* (2013) reported *D. pentandra* aqueous extract was potent towards T-47D breast cancer cell lines. Currently, there was no research that explained the mechanism of cell death of *D. pentandra* methanol extract against MCF-7 breast cancer cells, selectively with minimum effect towards normal cells. Thus,

*Corresponding Author
Mohd Dasuki Sul'ain (Ph.D.), Biomedicine Programme, School
of Health Sciences, Universiti Sains Malaysia, 16150 Kota Bharu,
Kelantan, Malaysia. E-mail: drdasuki @ usm.my*

this present study was performed to determine the potential of *D. pentandra* as an anticancer agent towards breast cancer as well as its antiproliferative property and mechanism of cell death.

MATERIAL AND METHODS

Plant collection and extraction

The *D. pentandra* leaves were collected from Pasir Hor, Kelantan, Malaysia and were authenticated by a botanist from School of Biological Sciences, Universiti Sains Malaysia (USM), Penang, Malaysia with voucher specimen number 11289. The leaves were dried in an oven at 50°C for 3 days. The extraction was *performed* as previously described with a *slight modification* (Zainuddin and Sul'ain, 2015). The *D. pentandra* was soaked in 100% methanol (Merck, Germany) with 1:10 ratio and left at room temperature for overnight. The mixture was filtered and the filtrate was concentrated using a rotary evaporator (Heidolph, UK) with settings of the water bath (Memmert, Germany) at 45°C for 2 hours. The dark green condensate obtained was dried in the fume hood (ERLA, Malaysia) until a constant weight was obtained. Percentage yield of *D. pentandra* methanol extract was calculated using formula as final weight of [*D. pentandra* methanol extract (g)/initial weight of *D. pentandra* dried powder (g)] × 100%.

Antiproliferative activity

HepG2 (liver cancer cells), U-2 OS (bone cancer cells), MCF-7 (breast cancer cells), MDA-MB-231 (breast cancer cells), HeLa (cervical cancer cells), MDCK (kidney normal cells) and L-929 (connective normal cells) were obtained from ATCC, USA. All cell lines were cultured in Dulbecco's Modified Eagles Medium (DMEM); supplemented with 5% of foetal bovine serum, 100 IU/ml of penicillin and 100 μg/ml of streptomycin using 25 cm² flasks, in 95% humidified incubator (Sheldon Shell Lab, USA) with 5% CO_2 at 37°C. All items were purchased from Gibco Laboratories, USA. The antiproliferative activity was carried out using the MTT Assay as reported by Zakaria *et al.* (2009). Cells were seeded at 5×10^4 cells/well in 100 μl of medium for overnight. The old medium was discarded before addition of 200 μl fresh medium. The *D. pentandra* methanol extract and tamoxifen (Sigma-Aldrich, USA), the positive control were dissolved in dimethyl sulfoxide (DMSO) (Nacalai Tesque, Japan). A range of 0.04 μg/ml to 100 μg/ml of both types of treatment were added to cells. Each well received 2 μl of each concentration from the serial dilution. The negative control group was cultured cells in medium with the addition of DMSO. The plates were incubated for 72 hours. Then, 50 μl of 2 mg/ml MTT solution (Amresco, USA) was added. The plates were incubated for 4 hours. Then, MTT solution was discarded and 200 μl of DMSO was added. Further incubation was performed for 30 minutes using orbital shaker (Heidolph, UK). The absorbance was read on a spectrophotometric microplate reader (Biorad Model 680, USA) at 570 nm. Dose-response curves were constructed to determine the IC_{50} values. A cell line with lowest IC_{50} value was selected for the rest of the subsequent assays.

Nuclear morphology staining

The staining was *performed* as previously described with a *slight modification* (Zakaria *et al.*, 2009; Zazali *et al.*, 2013). The cells were seeded overnight before the treatment in a sterile Petri dish that consisted of sterile microscope poly-L-sine adhesion slide and medium. The next day, the MCF-7 cells were treated with IC_{50} of *D. pentandra* methanol extract as well as IC_{50} of tamoxifen for 24, 48 and 72 hours. Then, the slides were washed with phosphate buffered saline (PBS) and dried at 50°C. Slides were fixed with cold methanol and then incubated with 30 μg/ml Hoechst 33258 (Sigma-Aldrich, USA) at room temperature for 1 hour in the dark room. Nuclear morphology of each different duration of treatment was examined under a fluorescent microscope (Leica, Germany) at 20× magnification.

Cell cycle analysis

The analysis was performed as described by Zakaria *et al.* (2009) and Zazali *et al.* (2013). Briefly, the MCF-7 cells were seeded overnight before the treatment in 25 cm² flasks. The cells were treated with IC_{50} of *D. pentandra* methanol extract and IC_{50} of tamoxifen for 24, 48 and 72 hours. At the end of incubation, cells were harvested and collected after centrifuged. All cells were fixed in cold 70% ethanol and gently vortexed for 1 hour at 20°C. Pellet of the cell was resuspended in 1% Triton X-100 (Amresco, USA) in PBS and 100 μl of 1 mg/ml RNase A (Amresco, USA). The mixture was kept at 37°C for half an hour. Cells were stained with 100 μl of 10 μg/ml propidium iodide (PI) (Sigm-Aldrich, USA) solution in staining buffer. Incubation was allowed at room temperature for 30 minutes. Then, sample tubes were kept in ice. Prior to flow cytometric analysis, 300 μl of staining buffer was added and analyzed using flow cytometry (Becton Dickinson FACS CANTO II, USA).

Measurement of apoptosis event

The quantification was performed as previously reported by Zakaria *et al.* (2009) and Zazali *et al.* (2013). The MCF-7 cells were seeded overnight before the treatment in 25 cm² flasks. The cells were treated with IC_{50} of *D. pentandra* methanol extract and IC_{50} of tamoxifen for 24, 48 and 72 hours. At the end of incubation, all cells were harvested and collected after centrifuged. The supernatant was discarded and the cell pellet was rinsed with DMEM. The tubes were spun at 666x×g for 5 minutes. The cell pellet was washed with cold PBS. The tubes were further centrifuged and resuspended in 1 X Assay Buffer. The cell suspension was added with Annexin V FITC/PI (Santa Cruz, USA) and incubated in dark room for 15 minutes. Samples were analyzed immediately within 1 hour using a flow cytometry after addition of 1 X Assay Buffer.

Detection of apoptotic protein

The analysis was performed as described by Zakaria *et al.* (2009) and Zazali *et al.* (2013) with slight modification. The MCF-7 cells were seeded overnight before the treatment in 25 cm² flasks. The cells were treated with IC_{50} of *D. pentandra* methanol extract and IC_{50} of tamoxifen for 24, 48 and 72 hours. At the end of incubation, all cells were harvested, collected and fixed in ice-cold 70 % ethanol for 1 hour at 20°C. Then, the cell pellet was washed twice with 1 X PBS and resuspended in blocking buffer with 2% bovine serum albumin (Amresco, USA) for 10 minutes. The cell pellet was resuspended in PBS to a concentration of 1×10^7 cells/ml and incubated with antibodies (Santa Cruz, USA) for 30 minutes in the dark at room temperature. The antibodies; Bax-

phycoerythrin (PE) conjugated, p53-PE conjugated and Bcl-2-PE conjugated were used in this study. The tubes were resuspended in 500 µl of 1 X PBS and analyzed by flow cytometry immediately.

Statistical analysis

Statistical analysis was performed using Graph Pad PRISM Version 6.0 GraphPad Software Inc, California, United States. Each experiment was conducted in triplicate and data were expressed as mean ± S.D. Statistical significances of data obtained were calculated and determined using Students' paired t-test or two-way ANOVA with Turkey's multiple comparison tests. The significance level of 0.05 ($p < 0.05$) was set for null hypothesis rejection.
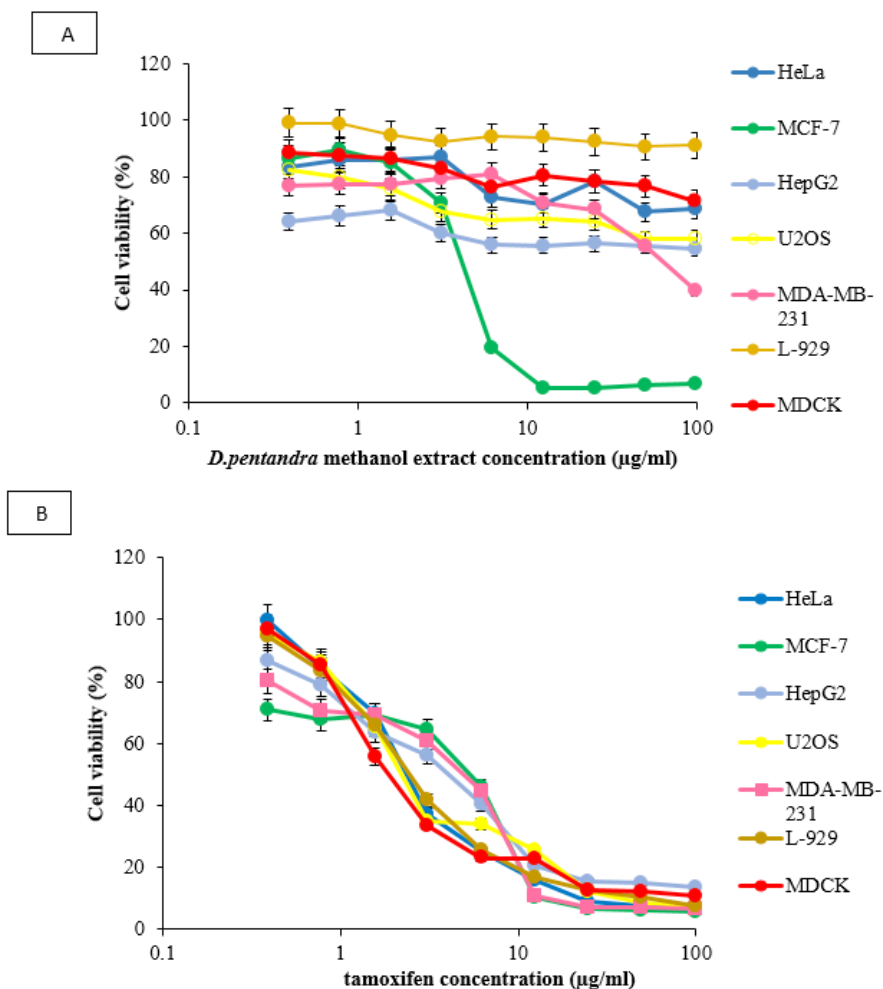

Fig. 1: Antiproliferative activity of *D. pentandra* methanol extract (A) and tamoxifen (B) on HeLa, HepG2, U2OS, MDA-MB-231, MCF-7, MDCK and L-929 cell lines. Each point showed the percentage of viable cells compared to negative control, DMSO. Each point represented as mean ± S.D, $n = 3$.

RESULTS AND DISCUSSION

Yield of extraction

Extraction by 100% methanol showed that 100 g of *D. pentandra* powder produced 35.8 g of *D. pentandra* methanol extract with 35.8% yield. Pattanayak *et al.* (2011) reported that methanolic extract of *Dendrophthoe falcata* produced only 12.32% yield which much lower than the yield in the current study.

Antiproliferative activity

The effect of *D. pentandra* methanol extract on cell proliferation was obtained from IC$_{50}$ value, the concentration of drug that caused inhibition of 50 % of cell survival,

compared to the drug, *in vitro* (Matic *et al.*, 2013). According to National Cancer Institute (NCI), a crude extract possessed a good antiproliferative activity if the IC$_{50}$ value was less than 20 µg/ml, following incubation period 48-72 hours (Boik, 2001). The preliminary screening of antiproliferative activity of *D. pentandra* methanol extract was performed to choose the most effective suppression growth of cancerous cells against *D. pentandra* methanol extract. Among five different types of cancerous cell lines (Figure 1A), *D. pentandra* methanol extract was most effective towards MCF-7 cells with an IC$_{50}$ value of 10.65 ± 0.49 µg/ml. There were no remarkable effects on non-malignant cells, L-929 and MDCK cell proliferation throughout all *D. pentandra* methanol extract treatment time. This synchronized with Zazali *et al.* (2013) report which

stated that IC_{50} more than 99 µg/ml on the proliferation of normal cells were considered harmless to the cells. Hence, *D. pentandra* methanol extract was less cytotoxic compared to *D. pentandra* ethyl acetate extract where the IC_{50} value was reported low as 18.12 ± 3.46 µg/ml on the proliferation of L-929 cells (Yee *et al.*, 2017). Tamoxifen showed an inhibitory effect where cytotoxic against tested cancerous cell line (Figure 1B) and also cytotoxic against normal cells. In contrast to tamoxifen, *D. pentandra* methanol extract showed no remarkable effect towards non-malignant cells and cytoselective against MCF-7 cell compared to another type of tested cancerous cells (Zazali *et al.*, 2013; Zainuddin and Sul'ain, 2015). Although *D. pentandra* methanol extract was less effective compared to tamoxifen in killing MCF-7 cells, it had lower cytotoxicity against the normal cells. Elimination of malignant cells without leaving any adverse effects on normal cells was regarded as apoptosis (Rahman *et al.*, 2013).

Nuclear morphological changes

Hoechst 33258 dye stained condensed nuclear of apoptotic cells and bound to packed regions of adenine and thymine in double-stranded deoxyribonucleic acid (DNA) to produce enhanced fluorescence (Zakaria *et al.*, 2009; Rahman *et al.*, 2013). In this study, there were obvious nuclear morphological changes in MCF-7 cells (Figures 2A, 2D, and 2G) after treatment with *D. pentandra* methanol extract which was indicative of apoptosis. The results obtained showed cell rounding, losing contact with neighboring cells, chromatin condensation and formation of apoptotic bodies were observed throughout the 24 hours to 72 hours of treatment with *D. pentandra* methanol extract. Tamoxifen-treated MCF-7 cells (Figure 2B, 2E, and 2H) displayed similar morphologically apoptotic events and nuclear-fluorescence in all treatment duration. The untreated MCF-7 cells (Figures 2C, 2F, and 2I) maintained in their shape, remained unstained and had a normal dull blue color nucleus which indicated the cells were not apoptotic cells (Zakaria *et al.*, 2009).
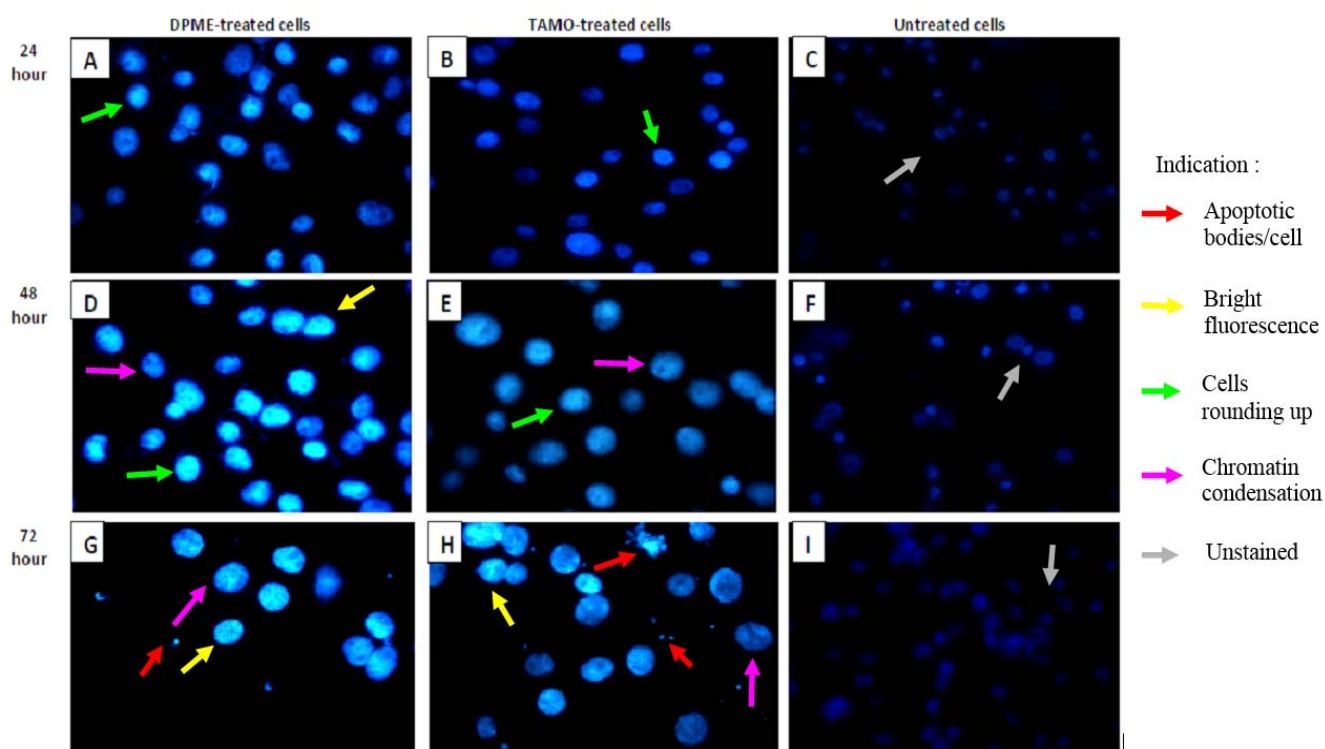

Fig. 2: Nuclear staining of MCF-7 cells by Hoechst 33258 dye. Cells were left untreated (C, F and I), treated with *D. pentandra* methanol extract (A, D, G) and 4.77 µg/ml with tamoxifen (B, E and H) for 24, 48 and 72 hours. Magnification: 20X.

Cell cycle analysis

Cell cycle arrest had been used as an indicator for the anticancer activity of drugs which led to inhibition of cell growth (Zakaria *et al.*, 2009). In order to determine cell cycle arrest upon treatment with *D. pentandra* methanol extract, PI was used to bind stoichiometrically nucleic acids, leading to fluorescence emission that is proportional to DNA content in cell death. The DNA contents were represented in three distinguishable stages of the cell cycle of G1, S and G2/M phases. The checkpoints for DNA damage were positioned before S phase, G1/S or after S phase,

G2/M (Zazali *et al.*, 2013). Upon treatment with *D. pentandra* methanol extract (Figure 3 and 4B), DNA content accumulated gradually from $66.29 \pm 1.20\%$ at 24 hours, $68.74 \pm 0.62\%$ at 48 hours and $69.08 \pm 0.89\%$ at 72 hours of G1 phase. After that, S phase took place with a reduced percentage of DNA contents compared to the untreated MCF-7 cells from $25.28 \pm 1.90\%$ at 24 hours, $28.28 \pm 1.49\%$ at 48 hours and $28.31 \pm 0.86\%$ at 72 hours ($p < 0.05$). The DNA contents were significantly reduced during G2/M phase with $8.45 \pm 1.15\%$ at 24 hours, $3.05 \pm 0.74\%$ at 48 hours ($p < 0.0001$) and $2.63 \pm 0.77\%$ at 72 hours ($p < 0.0001$). At

this point, the growth of MCF-7 cells was slowed down at a G1/S checkpoint by promoting the G1 arrest. This situation led to the cells being disturbed prior to entering the S phase to synthesis the DNA. Thus, the growth of MCF-7 cells during the G2 phase of cell division was approaching towards termination. The termination of cell cycle progress in MCF-7 cells might be stimulated by DNA damage that had been triggered by *D. pentandra* methanol extract treatment. As reported by Widowati *et al.* (2013), the apoptotic cells were obtained by sub-G1 area from cell cycle analysis of 10% *D.*

pentandra aqueous extract induced apoptosis in a T-47D cell line with 24.025% of apoptotic rate. The data was also in accordance with previous findings by Endharti *et al.* (2016) that reported *D. pentandra* ethanol extract could prevent colonic epithelial cells proliferation by inhibition of S phase caused the G1 arrest of cell cycle. Treatment with tamoxifen showed a suppression effect on MCF-7 cells by arresting cell cycle progression as the DNA content during G1 phase increased dramatically and the low peak of G2/M was observed.
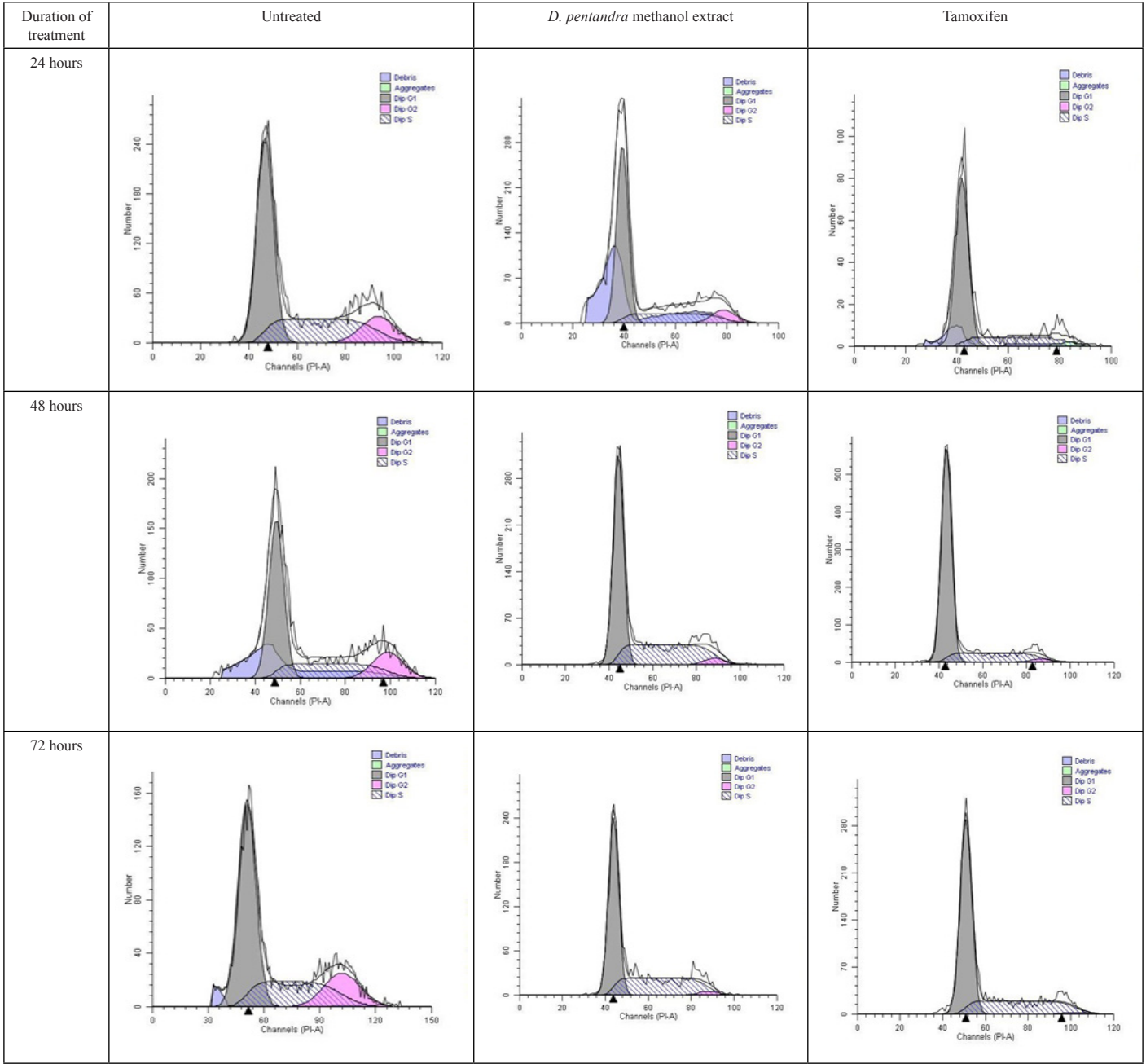

Fig. 3: Histogram profile of cell cycle progression in untreated MCF-7 cells, *D. pentandra* methanol extract -treated MCF-7 cells and tamoxifen-treated MCF-7 cells for 24 hours, 48 hours and 72 hours. Indication: ▣ Debris ▣ G1 ▣ G2 ▨ S.

Measurement of apoptosis event

      To further confirm the mechanism of *D. pentandra*

methanol extract induced apoptosis in MCF-7, the cells were double - stained with Annexin V-FITC and PI. An early sign of

apoptosis could be identified by exposure of phosphatidylserine (PS) to the outer layer of cell plasma membrane (Zazali *et al.*, 2013). During early apoptosis, PS were translocated from inner side of cell plasma membrane to outer side, allowing the exposure to be detected by Annexin V, a calcium-dependent phospholipid binding protein that had a high affinity for PS and this could be used for the detection of apoptosis (Arur *et al.*, 2003). Simultaneously, nuclear dye, PI has been probed to examine damage of cell membrane and stained the damaged DNA of cells at their late stages of apoptosis (Zazali *et al.*, 2013; Yaacob *et al.*, 2013). By this condition, apoptotic cells and necrosis cells could be distinguished.
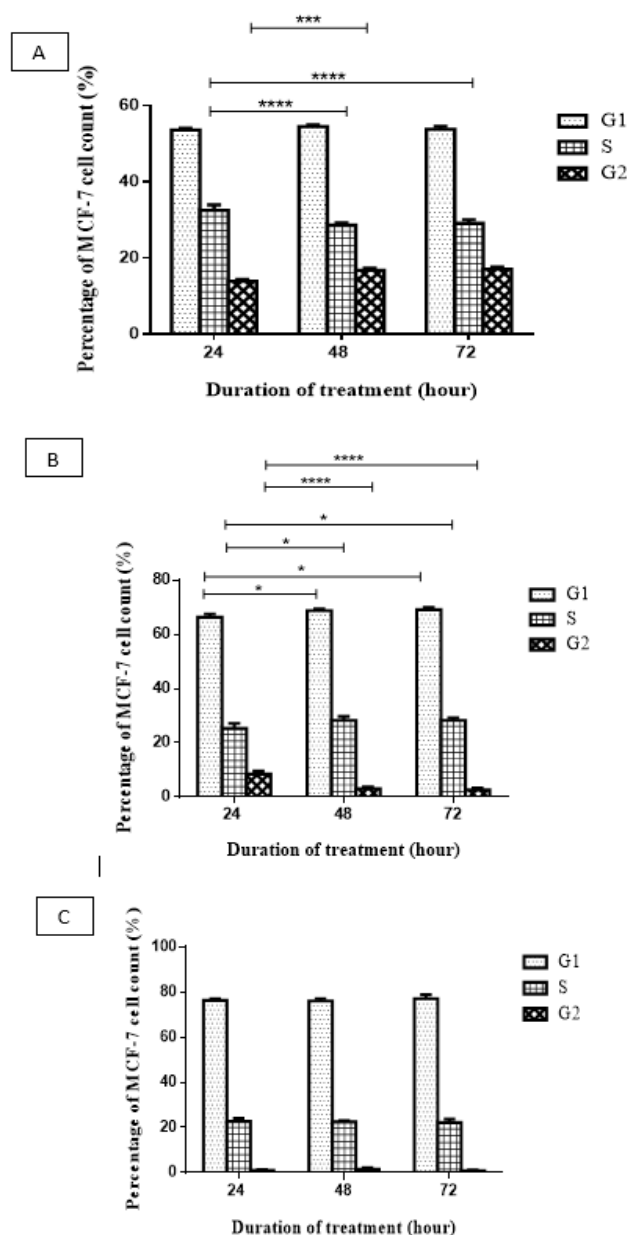

Fig. 4: DNA contents of MCF-7 cells for untreated (A), *D. pentandra* methanol extract (B) and tamoxifen (C), at each different treatment time. The value of bars represented mean ± S.D. of three independent experiments, with ****$p <$ 0.0001 and ***$p <$ 0.001 were taken as extremely significant different, and *$p <$ 0.05 was taken as significantly different with the treatment durations.

In *D. pentandra* methanol extract-treated group (Figure 5 and 6B), the number of viable cells (Q3) was less compared to untreated cells ($p <$ 0.0001) from 66.67 ± 1.45% to 28.6 ± 1.21% and 3.17 ± 0.35% during 24, 48 and 72 hours of treatment, respectively. This decrement showed that MCF-7 cells were induced to undergo cell death after being treated with *D. pentandra* methanol extract. The percentage of early apoptotic cells (Q4) was initially increased 18.43 ± 1.46% to 61.63 ± 0.75% and up to 83.63 ± 1.02% when incubated for 24, 48 and 72 hours ($p <$ 0.0001). This proved that apoptosis happened gradually and took place in MCF-7 cells within 48 hours since more than 50% of cell population undergo early apoptosis event. Wong *et al.* (2013) also reported similar results in breast cancer cells where *Vernonia amygdalina* extract induced early apoptosis after 24 hours of treatment in a time-dependent manner, compared to control.

The percentages of cell count detected at late apoptosis quadrant (Q2) for *D. pentandra* methanol extract were 12.37 ± 1.46%, 7.07 ± 0.75% and 11.47 ± 0.78% throughout 24 hours, 48 hours and 72 hours, respectively. This was significantly different ($p <$ 0.0001) when compared throughout treatment time. Some PS of cells still exposed to an outer layer of cell membranes, but consequently, most of the cell membranes were already affected by the treatment. Hence, cell membranes also became permeable towards PI. The percentage of the cell population at necrosis quadrant (Q1) remained low at 2.57 ± 0.12%, 2.7 ± 0.53%, and 1.73 ± 0.60% during all incubation periods. The dead cells were necrosis where PS of necrotic cells were not exposed to the outer layer of cells membrane. At this point, the whole cells were already affected and ruptured. This released the DNA and PI could easily bind to necrotic cells. A small population of cells had undergone necrosis and late apoptosis where the cell membranes lost their integrity as shown in the *D. pentandra* methanol extract-treated tamoxifen-treated group at 72 hours incubation time.

To extend the above findings, the mechanism of apoptosis in *D. pentandra* methanol extract-treated MCF-7 cells was further analyzed by evaluation of apoptotic proteins p53 (Figure 7), Bax (Figure 8) and Bcl-2 (Figure 9). These proteins were among the important models to determine the status of the apoptosis pathway (Jourdain and Martinou, 2009; Zakaria *et al.*, 2009; Zazali *et al.*, 2013). In *D. pentandra* methanol extract-treated group (Figure 10B), level of p53 protein was increased gradually throughout the treatment period ($p <$ 0.0001) where 21.63 ± 1.33%, 66.00 ± 2.93% and 75.60 ± 1.41% were shown at all treatment period, respectively. By comparing with untreated groups, p53 expression showed significant difference with $p <$ 0.0001 when treated with *D. pentandra* methanol extract at 72 hours. The expression of Bax proteins was higher as early as 24 hours of post-incubation treatment with 84.43 ± 2.51%. Slightly increased in Bax expression compared to untreated cells to 90.40 ± 1.35% and 91.60 ± 1.3% were observed at 48 hours and 72 hours, respectively. However, the Bcl-2 protein level decrease ($p <$ 0.0001) to 66.4 ± 1.25%, 52.00 ± 1.61% and 15.90 ± 0.80% throughout 24, 48 and 72 hours, respectively.

Consequently, p53 had influenced the induction of the apoptosis pathway in *D. pentandra* methanol extract. The ability of p53 to express in *D. pentandra* methanol extract treatment was dependent on p53 protein. In conjunction with the above findings, Bax production was believed to be activated by p53 (Zakaria *et*

al., 2009) that highly accumulated in MCF-7 cells. p53 might be responsible for the mechanism of *D. pentandra* methanol extract-treated cells where this condition was proved by the ability of p53 to downregulate Bcl-2 and up-regulate Bax before proceeding to apoptosis pathway (Meenakshii *et al.*, 2000; Abdullah *et al.*, 2010). Acting as a transcription factor, p53 helped to promote apoptosis by transcription of its target genes of Bcl-2 family members. p53 was a widely studied cancer suppressor and its response to diverse forms of cellular stress to mediate a variety of antiproliferative processes has been proven (Abdullah *et al.*, 2010) and has been termed 'the guardian of the genome'. This was due to its pivotal role in managing the integrity of genetic information in response to various genotoxic injuries (Wong *et al.*, 2013). In mammalian cells, there was a notable G1 arrest that seems to be associated by

tumor suppressor p53 (Hung *et al.*, 1996). The above finding was in accordance with our earlier data that the *D. pentandra* methanol extract-treated cell stopped cell cycle at G1/S has activated the p53 protein. It was also supported by Endharti *et al.* (2016) where the G1 arrest of the cell cycle upon treatment with *D. pentandra* ethanol extract was through induction of p53 expression. The efficacy of tamoxifen in this study was also in accordance with Kamal (2014) finding which reported that there were increased p53 expression after 24 hours treatment and remained persistently high after 48 hour treatment towards MCF-7 cells. The apoptotic effect of tamoxifen was associated with some modulation in the expression of *Bcl-2* family proteins such as Bax and Bcl-2 (Kallio *et al.*, 2005).
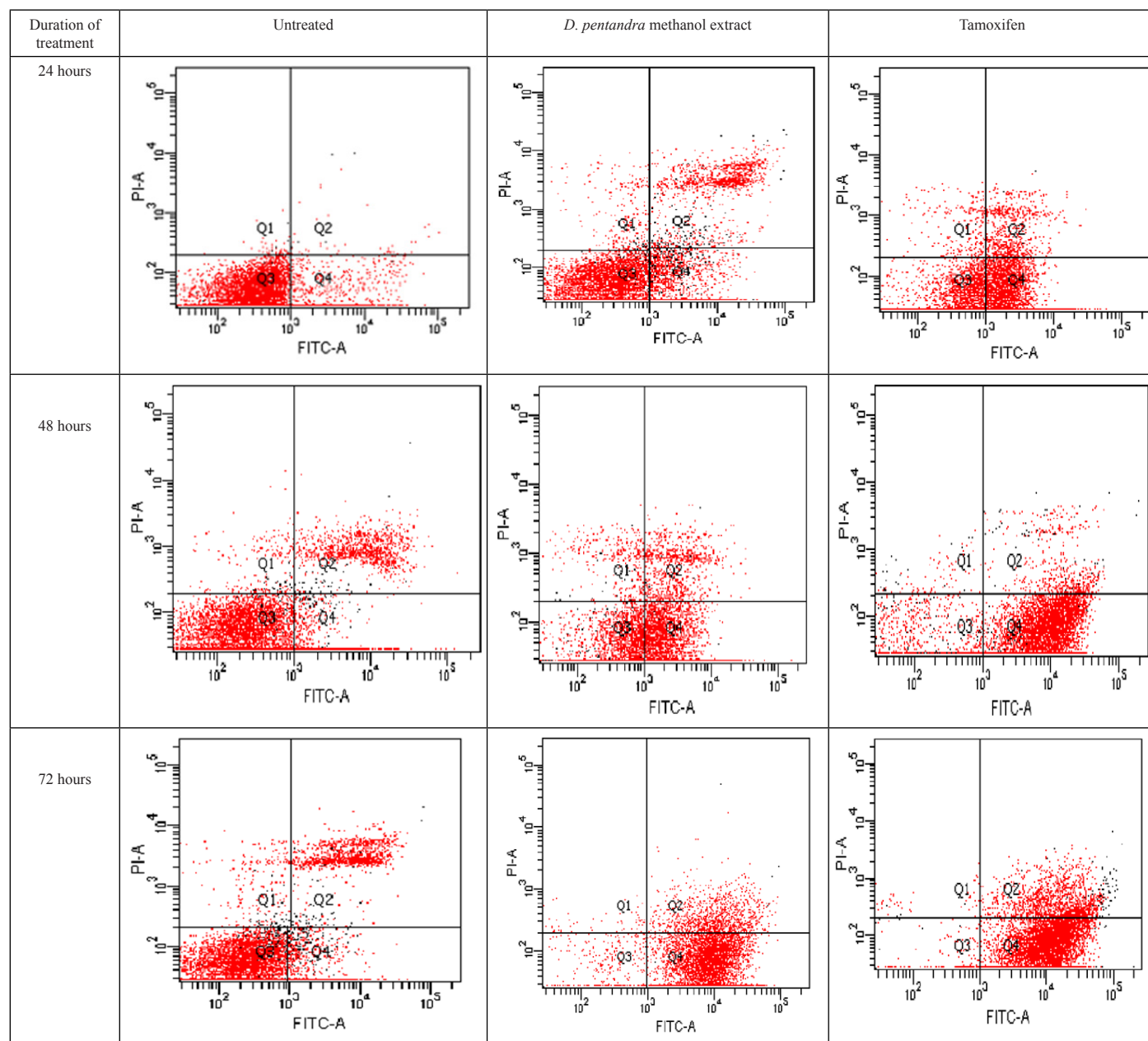

Fig. 5: Scatter plots of MCF-7 cells stained with PI/FITC under four quadrant analysis; viable cells (Q3), early apoptotic cells (Q4), late apoptotic cells (Q2) and necrotic cells (Q1) in untreated MCF-7 cells, *D. pentandra* methanol extract-treated MCF-7 cells and tamoxifen-treated MCF-7 cells for 24, 48 and 72 hours.
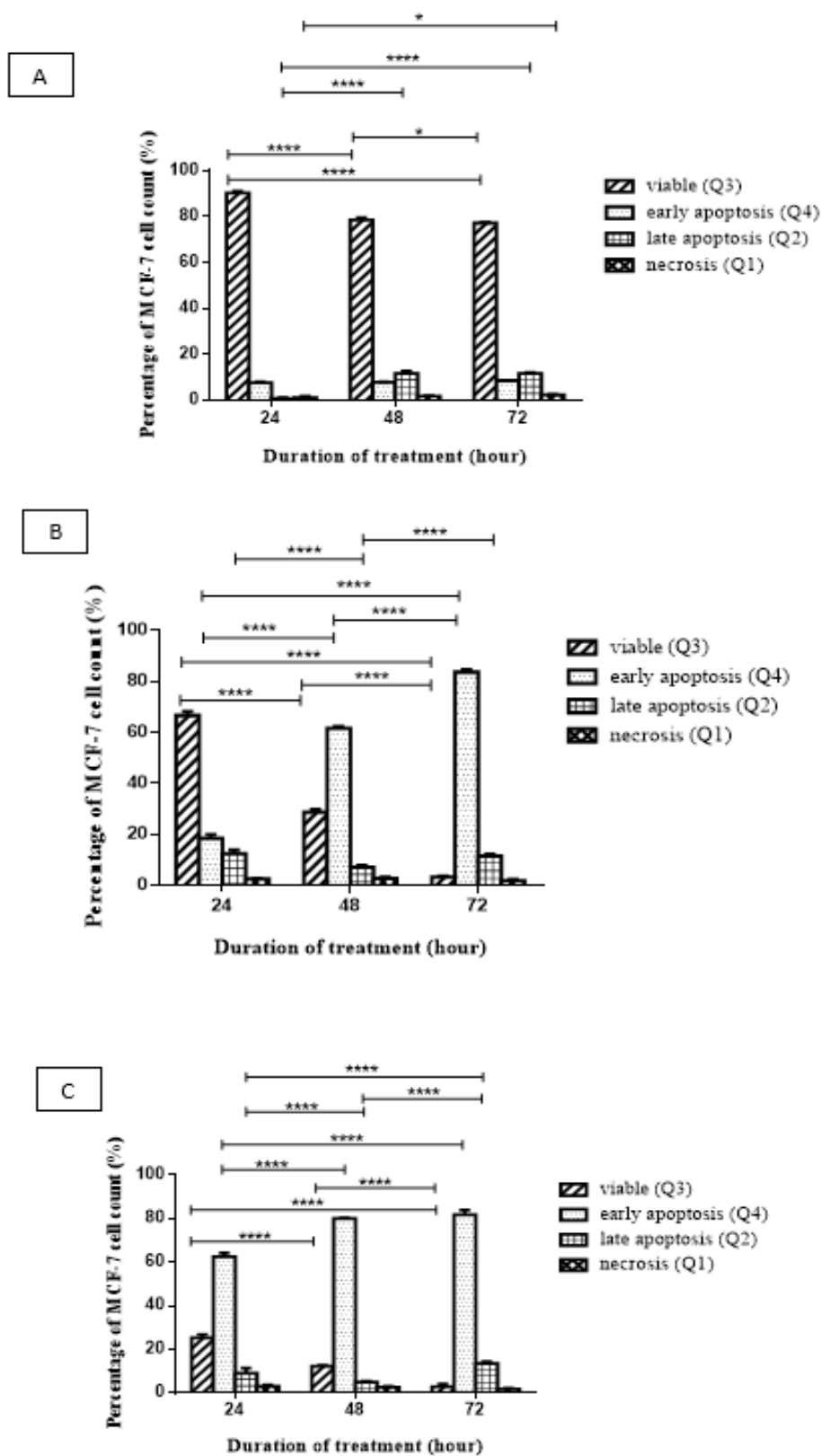
Fig. 6: Cell count of MCF-7 cells for untreated (A), *D. pentandra* methanol extract (B) and tamoxifen (C) at each different treatment time. The value of bars represented mean ± S.D. of three independent experiments, with ****$p < 0.0001$ was taken as extremely significant different and *$p < 0.05$ was taken as significantly different with the treatment durations.
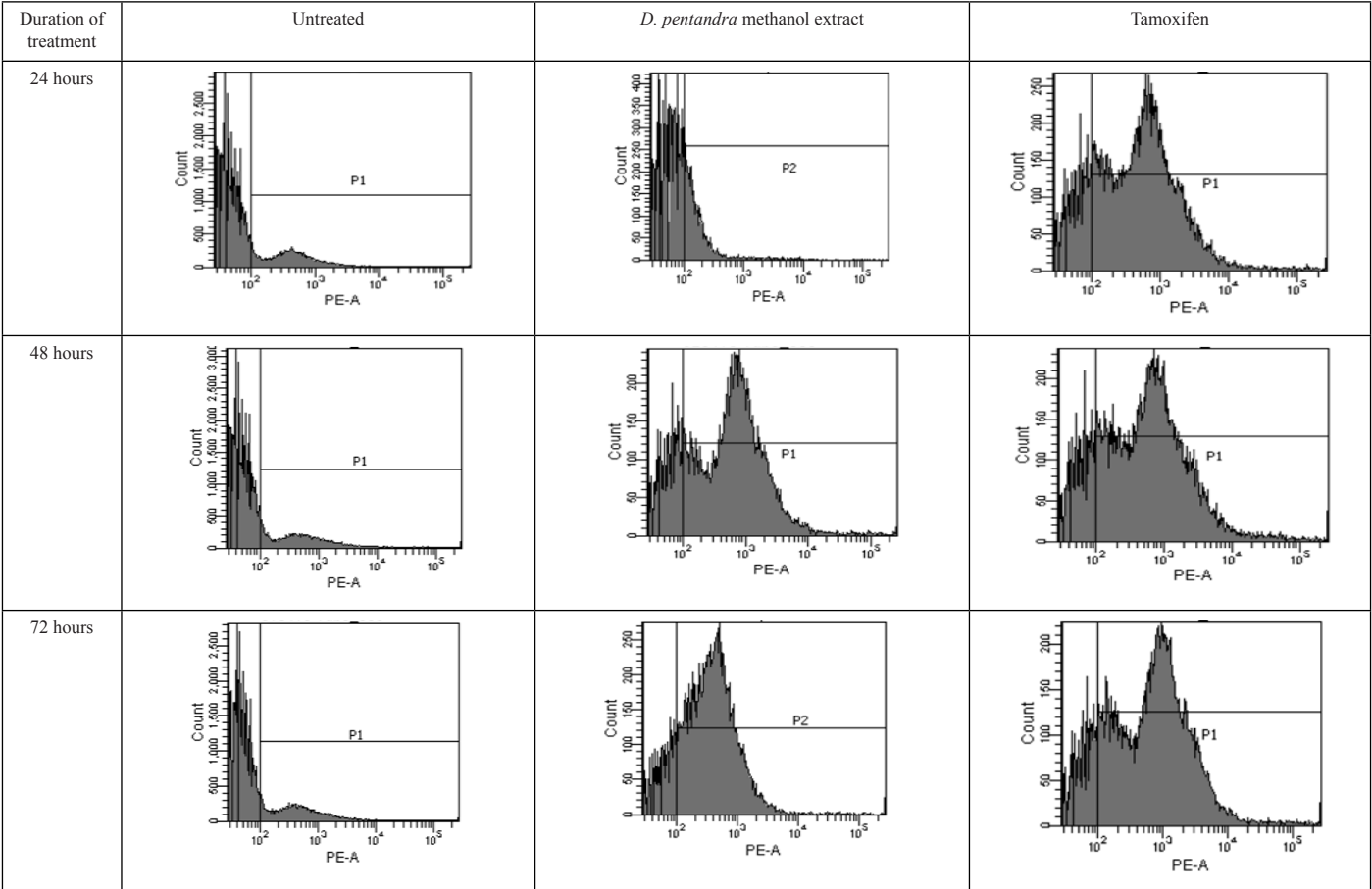
Detection of apoptotic protein

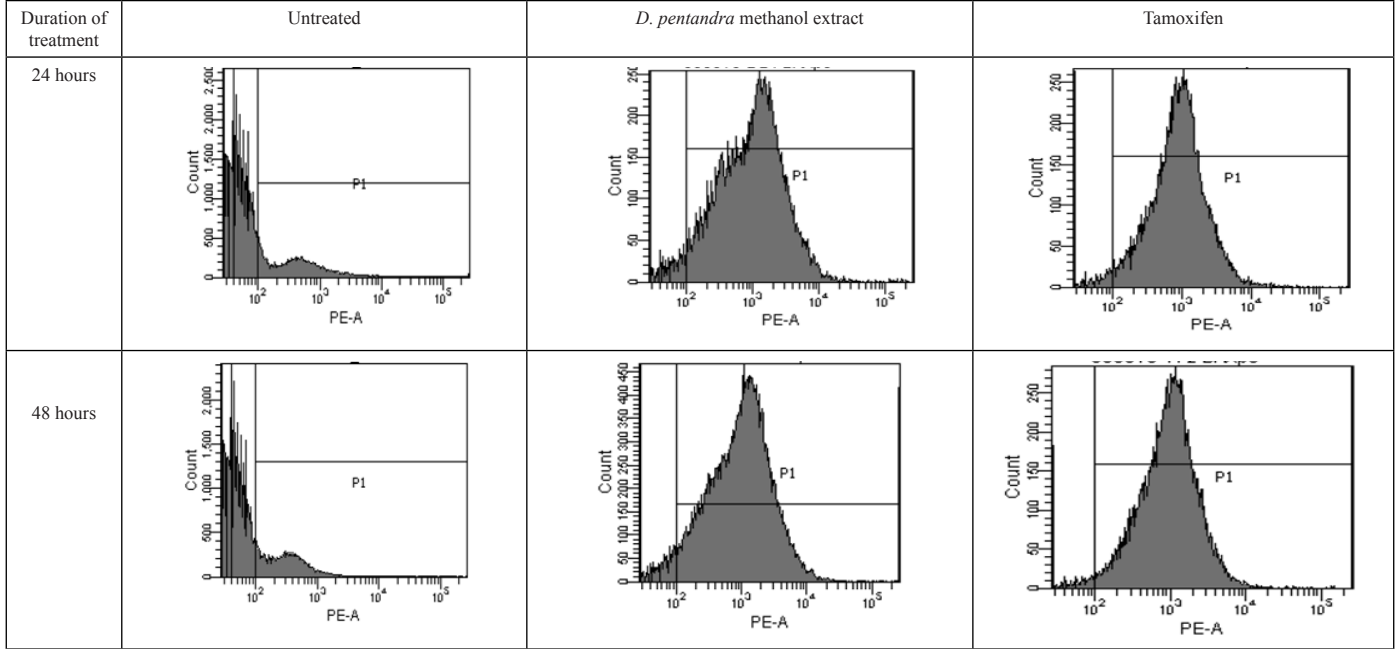| Duration of treatment	Untreated	*D. pentandra* methanol extract	Tamoxifen
24 hours			
48 hours			
72 hours			

Fig. 7: Histogram profile of p53 proteins expression in untreated MCF-7 cells, *D. pentandra* methanol extract-treated MCF-cells and tamoxifen-treated MCF-7 cells for 24 hours, 48 hours and 72 hours.
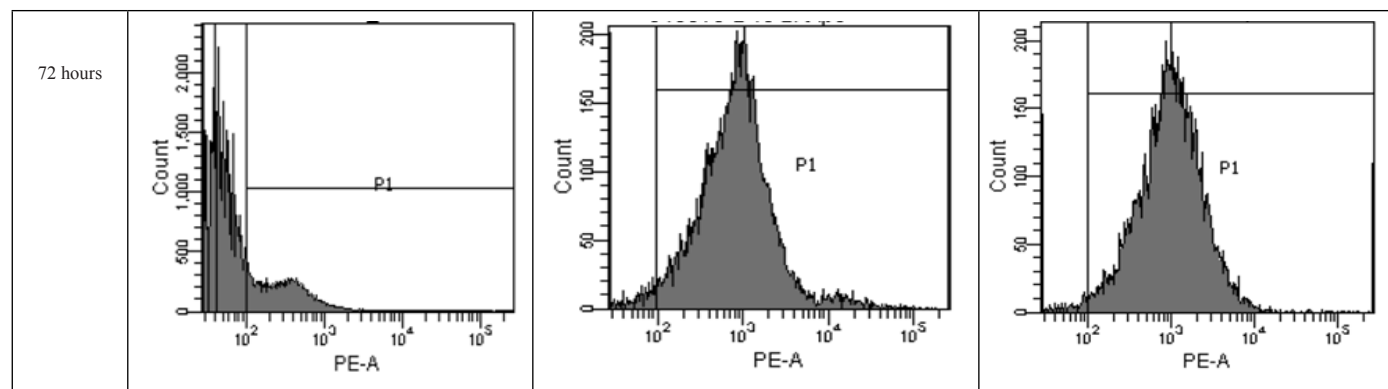
Duration of treatment	Untreated	*D. pentandra* methanol extract	Tamoxifen
24 hours			
48 hours			

Fig. 8: Histogram profile of Bax proteins expression in untreated MCF-7 cells, *D. pentandra* methanol extract-treated MCF-cells and tamoxifen-treated MCF-7 cells for 24 hours, 48 hours and 72 hours.
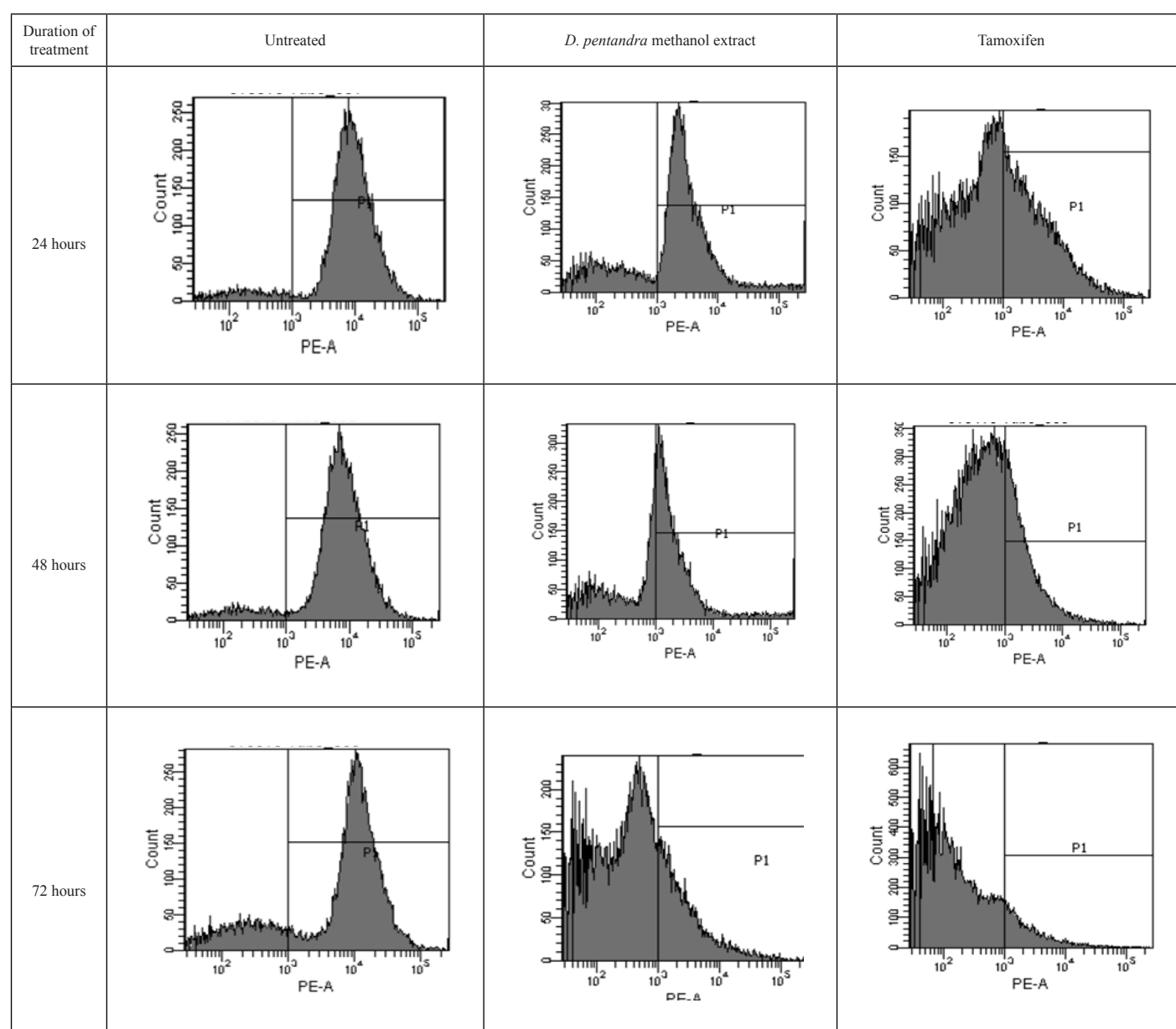

Fig. 9: Histogram profile of Bcl-2 proteins expression in untreated MCF-7 cells, *D. pentandra* methanol extract-treated MCF-cells and tamoxifen-treated MCF-7 cells for 24 hours, 48 hours and 72 hours.
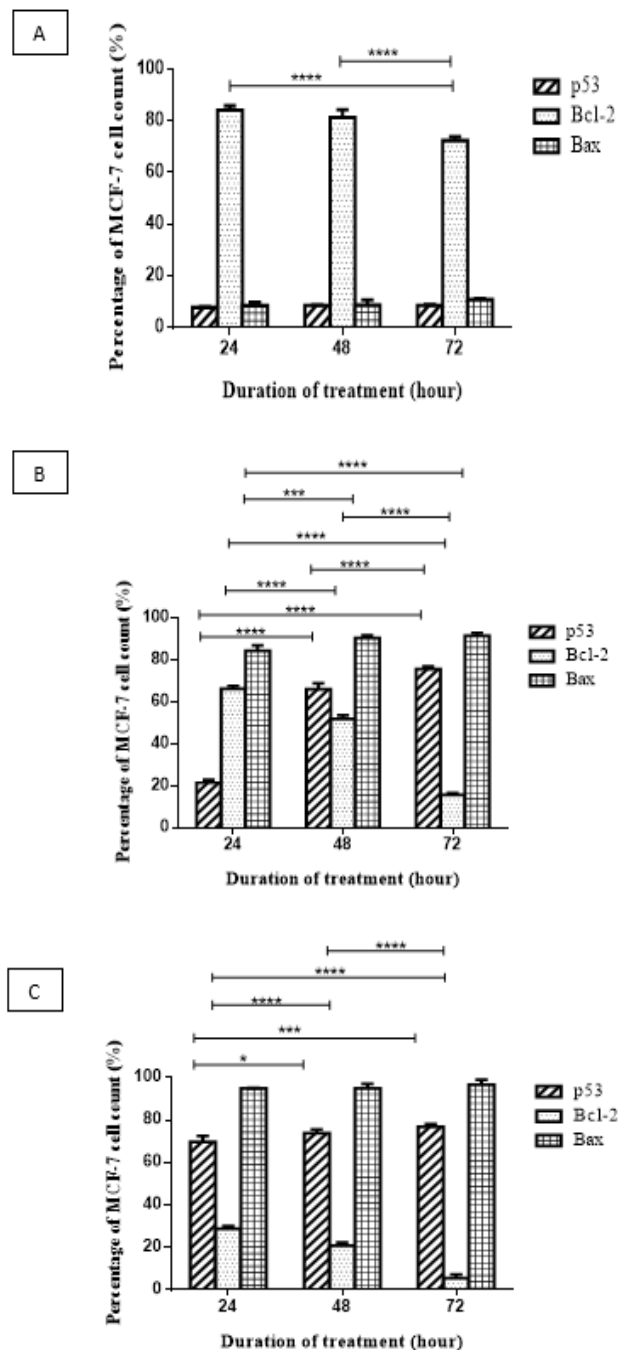
Fig. 10: Cell count of each expression levels of p53, Bcl-2 and Bax for untreated (A), *D. pentandra* methanol extract (B) and tamoxifen (C) at each different treatment time. The value of bars represented mean ± S.D. of three independent experiments, with ****$p < 0.0001$ and ***$p < 0.001$ were taken as extremely significant different and *$p < 0.05$ was taken as significantly different with the treatment durations.

CONCLUSION

       D. pentandra methanol extract was selective against MCF-7 breast cancer cells and showed no remarkable negative effect on normal cells. Typical morphologies of apoptosis cell death were displayed throughout all treatment period with *D. pentandra*

methanol extract. The growth of MCF-7 cells was arrested at a G1/S checkpoint. This was associated with the help of p53 throughout the apoptosis pathway. In this study, apoptosis was shown as a major cell death mechanism as demonstrated by *D. pentandra* methanol extract which induced early apoptosis in MCF-7 cells. Along with the role of p53, the apoptosis pathway also was promoted by activation of Bax and inactivation of Bcl-2. The findings from this study proved that *D. pentandra* methanol extract was able to inhibit the proliferation of MCF-7 cells by induction of apoptotic cell death. Thus, *D. pentandra* methanol extract demonstrated potential anticancer activities in MCF-7 cells by apoptosis induction.

CONFLICTS OF INTEREST

       The authors do not have any conflicts of interest regarding the content of the present work.

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