Journal of Medicinal Plants Research Vol. 5(24), pp. 5741-5749, 30 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Elephantopus scaber induces cytotoxicity in MCF-7 human breast cancer cells via p53-induced apoptosis

Wan Yong Ho[1], Swee Keong Yeap[2], Chai Ling Ho[1], Abdul Rahim Raha[1,2], Abdul Aziz Suraini[2,3] and Noorjahan Banu Alitheen[1,2]*

[1]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang 43300 Selangor, Malaysia.
[2]Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor Malaysia.
[3]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang 43300 Selangor, Malaysia.

Accepted 12 September, 2011

Plants have not only been consumed as food but have also been adopted as folk medicine for centuries. *Elephantopus scaber* Linn, a herb from the Asteraceae family, has traditionally been taken as decoction or tea to cure various ailments and diseases throughout the world. Recent studies had also suggested that this plant posses various bioactivities such as anti-bacterial, anti-inflammatory, hepatoprotective as well as anti cancer properties. In this study, the cytotoxic effect of an ethanolic extract of *E. scaber* on a breast cancer cell line, MCF-7 and the underlying cell death mechanism was examined. *E. scaber* showed cytotoxic effect towards MCF-7 cells with an IC_{50} value of 15 µg/mL. In comparison to the untreated control, the extract triggered cell death with increased phosphatidylserine externalization, DNA breaks and significant morphological apoptotic characteristics in the MCF-cells. Furthermore, we also found that expression of the tumor suppressor p53 protein was up-regulated in response to the treatment. In conclusion, these results suggested that the ethanolic extract of *E. scaber* may be a potential anti cancer agent for human breast cancer cells by the induction of p53-dependent apoptosis.

Key words: Ethanol extract, MCF-7, tumor suppressor protein, DNA fragmentation, phosphatidylserine externalization.

INTRODUCTION

Breast cancer is one of the leading causes of cancer mortality worldwide every year. Despite the availability of treatments in the form of surgery, radiation therapy, chemotherapy, hormonal therapy and biologic therapy for breast cancers (American Cancer Society, 2007; World Health Organization, 2011), most of the therapeutic means are associated with some drawbacks such as high cost of treatment and adverse side effects after prolonged exposure. For instance, tamoxifen, the oldest and most-prescribed selective estrogen receptor modulator (SERM) for treating hormone-receptor-positive breast cancer, had been proven to reduce the risk of developing invasive and non-invasive breast cancer among women (Fisher et al., 2005). However, clinical cases had also witnessed the susceptibility of women who underwent tamoxifen therapy for more than five years to have relatively higher risks for stroke, cataracts, cardiac arrhythmia or atrial fibrillation, hypertriglyceridemia, deep-vein thromboembolic events and even death (Fisher et al., 2005; Veronesi et al., 2007). There is indeed a need to search for more easily available and much more reliable therapeutic sources into overcoming the problems associated with current breast cancer treatments.

Natural products have been used for a long time in folk medicine to treat a great variety of diseases. In ethnopharmacology, plant-derived products are not only useful as medications to cure common injuries and ailments but are also well known for preventing and treating dreadful diseases such as cancers (Mann, 2000; Yeap et al., 2010). The ethnopharmacological evidence of natural products usage has therefore become an important lead for the discovery of anticancer drugs

*Corresponding author. E-mail: noorjahan@biotech.upm. edu. my. Tel: +603-89467471.

(Shelley, 2009). In modern medicine, herbal products are used widely but not limited to in many developed countries as a major complementary/alternative medicine (CAM) (Ernst, 2000; Molassiotis et al., 2005). The wide acceptance of traditional Chinese medicine in the Western countries has also popularized herbs as an alternative for cancer therapy (Chiaramonte and Lao, 2010).

Elephantopus scaber Linn, or more commonly known as Elephant's foot, is a perennial herb from the Asteraceae family that can be easily found in tropical countries. The whole plant of this herb can be consumed for the treatment of various ailments and diseases (Gurib-Fakim et al., 1993; Inta et al., 2008; Ho et al., 2009). Traditionally, *E. scaber* is used for treating diabetes, enteritis, flatulence, diuresis and is also adopted as an analgesic, astringent and antiemetic agent (Ong and Nordiana, 1999; Daisy et al., 2007; Lee et al., 2008). In India's Ayurvedic medicine, this herb, along with a few others is also used to treat neoplasm of different stages (Balachandran and Govindarajan, 2005). Nevertheless, there is indeed scientific evidence that has proved the antineoplastic activity of *E. scaber*. This plant showed anti proliferative activities towards carcinoma cell lines from the renal, liver, cervix and colon *in vitro* and inhibited the growth of a human cervical cancer tumor *in vivo* (Than et al., 2005; Ichikawa et al., 2006; Xu et al., 2006; Liang et al., 2008; Liang and Min, 2002). However, the lack of in depth study has limited our understanding of the cell death mechanism induced by *E. scaber* in human cancers. Therefore, in this study, the potential cytotoxic effect of *E. scaber* on the human breast cancer MCF-7cell line was evaluated by elucidation of the cell death mechanisms and regulation of tumor suppressor protein underlying the cytotoxicity of this herb.

MATERIALS AND METHODS

Preparation of *E. scaber* ethanol extract

E. scaber used in this work was collected from Georgetown, Penang, and was identified by science officer Mr. Lim Chung Lu from the Forestry Division of Forest Research Institute Malaysia (FRIM), Kepong, Selangor. The voucher number deposited in FRIM for *E. scaber* is FRI65693. The leaves of *E. scaber* were thoroughly washed, shade dried and ground into fine powder. The leaf powder was then subjected to extraction for three times with absolute ethanol at room temperature. All the extracts were then mixed and filtered through grade 1 Whatman filter paper with subsequent evaporation to dry under reduced pressure using Aspirator A-3S (EYELA, Japan) at < 40°C. The final yield was 8% of the initial dried leaves' weight. A stock of the extract was prepared by dissolving the powder in DMSO at a concentration of 10 mg/ml and it was stored at 4°C.

Cell lines and cell culture

Human breast carcinoma MCF-7 and human breast cell control MCF-10A was purchased from the American Type Culture Collection (ATCC, Manassas, VA). MCF-7 was routinely cultured in RPMI-1640 (Sigma) while MCF-10A was maintained in a 1:1 mixture of DMEM and F12 medium (DMEM-F12) supplemented with 5% (v/v) horse serum, hydrocortisone (0.5 µg/ml), insulin (10 µg/ml) and epidermal growth factor (20 ng/ml). All media were supplemented with 10% (v/v) heat-inactivated foetal bovine serum, penicillin (100 U/ml) and streptomycin (50 U/ml). The cells were cultured at 37°C in a humidified incubator with 5% CO_2.

MTT cytotoxicity assay

The cytotoxic potential of ESEE against MCF-7 and MCF-10A cell lines was determined via the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as previously described (Mosmann, 1983). In brief, the cells that were cultured overnight in 96-well plates were treated with fresh media containing different concentrations of ESEE ranging from 1.56 to 100 µg/mL (Tamoxifen with concentrations from 0.47 to 100 µg/mL was used as positive control for MCF-7 cell line). After further incubation of 24, 48 and 72 h, the absorbance was recorded at 570nm in a µ Quant ELISA Reader (Bio-tek Instruments, USA). Triplicates were carried out for all cell lines. Percentage of proliferation was calculated using the following formula:

$$\text{Percentage of cell viability} = \left(\frac{\textbf{OD sample}}{\textbf{OD control}}\right) X\ 100\%$$

Cells seeding and treatment

MCF-7 cells were used for the acridine orange and propidium iodide dual staining, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (Tunel) assay, phosphatidylserine externalization analysis and protein phenotyping. In all these assays, the cells were seeded at a concentration of 2 X 10^5 cells/ well in 6-well plates for 24 h prior to treatment. On the following day, RPMI medium was discarded and replaced with 2ml of fresh medium in each well. The cells were subjected to treatment by 15.0 µg/ml of *E. scaber* ethanol extract (ESEE). After incubation for 24, 48 and 72 h, the control and treated cells were detached using recombinant trypsin (TrypLE™ Express, Gibco) and spun down by centrifugation (1000 rpm for 10 min) before proceeding to the corresponding assays.

Acridine orange and propidium iodide double staining

Apoptosis was determined by staining MCF-7 cells with acridine orange (AO) and propidium iodide (PI). The harvested cells were dissolved in 100 µl of PBS. Then, staining dyes (10 µg/ml of AO and PI each) were added into the cell suspension and 10 µl of the stained cells were immediately observed under an inverted fluorescent microscope (Nikon, Japan).

Tunel assay

Tunel assay was carried out according to the protocol of the APO-DIRECT™ kit (Becton Dickinson, USA). In brief, the harvested cells were suspended in 1% (w/v) paraformaldehyde in PBS (pH 7.4). The cell suspension was placed on ice for 60 min followed by centrifugation at 300 x g. Pellet was then washed twice with PBS. Cell concentration was adjusted to 1 x 10^6 cells per ml of 70% (v/v) ice cold ethanol and let to stand for 1 h on ice before storing at -20°C. After 24 h of incubation, the cells were stained according to

Table 1. IC_{50} values of MCF-7 and MCF-10A upon treatment with ESEE and tamoxifen.

Treatment	Cancer cell type	Cell line	Average mean values of IC_{50} (µg/mL)		
			24 H	48 H	72 H
ESEE	Human breast carcinoma	MCF-7	14.69 ± 0.29	7.33 ± 1.20	7.17 ± 1.09
	Human normal breast cell	MCF-10A	> 80	> 80	> 80
Tamoxifen	Human breast carcinoma	MCF-7	11.6 ± 0.3	9.6 ± 0.65	10.2 ± 0.45

the protocol for measurement of apoptosis and then subjected to analysis using the FACS Calibur flow cytometer.

Phosphatidylserine (PS) externalization

The annexin V method was used to investigate PS externalization. Cells were washed twice with cold PBS followed by double staining with Annexin V-FITC) and propidium iodide (PI) using the FITC Annexin V Apoptosis Detection Kit' according to the manufacturer's protocol (Becton Dickinson, USA). Data acquisition and analysis were performed in FACS Calibur flow cytometer (Becton Dickinson, USA) using CellQuest 3.3 software.

p53 monoclonal antibody staining

The fixation and staining of cells with p53 monoclonal antibody was carried out according to the protocol of Cytofix/Cytoperm™ Fixation/Permeabilization Kit (Becton Dickinson, USA). First, 250 µl of fixation/permeabilization solution was added into each of the harvested cell pellet and incubated at 4°C for 20 min. Then, the cells were washed twice in BD Perm/Wash™ buffer. For staining of cells, the cells were resuspended in 50 µl of BD Perm/Wash™ buffer containing the p53 monoclonal antibodies followed by 30 min of incubation at 4°C in the dark. The cells were then washed twice and further conjugated with FITC Goat Anti-Mouse IgG and incubated for another 30 min at 4°C in the dark. The cells were washed twice and resuspended in staining buffer prior to flow cytometric analysis.

Statistical analysis

Results were expressed as mean ± standard error (S.E.M.). Three separate experiments were performed for each measurement. Differences between the groups were evaluated by using ANOVA test (one way) followed by Duncan test and $p \leq 0.05$ was taken as being statistically significant.

RESULTS AND DISCUSSION

Despite the increasing amount of marketed antineoplastic drugs, a huge population of the world is still using herbs as a mean for cancer therapy. Previous researches had shown that *E. scaber* exerted differential cytotoxic effects towards a few cancer cell lines (Than et al., 2005; Ichikawa et al., 2006; Xu et al., 2006; Liang et al., 2008; Liang and Min, 2002). However, most studies involving cytotoxicity of its compounds were limited to screening of their anti proliferative activities with no further evaluation on the cell death mechanism. We were thus attempted to examine further the cytotoxic and apoptotic properties of ESEE in MCF-7 cells. The MTT assay was carried out to assess the cytotoxicity of ESEE on MCF-7 and a normal human breast cell line MCF-10A. The IC_{50} value, concentration of the extract that causes 50% growth inhibition or 50% cell kill of the cell lines was determined from this assay. Table 1 shows the mean average IC_{50} profiles of ESEE against these two cell lines. It was apparent that progressively longer exposure of these cell lines to ESEE resulted in a lower extract concentration needed to achieve IC_{50} in MCF-7 but the IC_{50} value of MCF-10A remained higher than 80 µg/mL after all three incubation periods. In addition, tamoxifen was used as a positive control for MCF-7 cell line. The IC_{50} value of MCF-7 treated with ESEE was higher than tamoxifen after 24 h but its effect prevailed that of tamoxifen with lower IC_{50} values after 48 and 72 h of incubation.

On the other hand, screening of ESEE on the normal human breast cell line, MCF-10A revealed that ESEE did not cause cytoxicity in this positive control of normal human breast cell line, at least at a concentration that was much higher than its IC_{50} concentration on MCF-7. This could thereby suggest that ESEE could induce cytotoxicity in breast cancer cells without affecting the normal breast cell population, therefore potentiates ESEE as a safe treatment for human breast cancer. Direct observation of the morphological changes of MCF-7 cells revealed growth inhibition and induction of cell death after treatment with ESEE. Microscopic images of the cells could be observed clearly from the cell culture plates as shown in Figure 1. Membranes of the detached cells remained intact even after incubation for 72 h with 15 µg/mL of the extract, suggesting that ESEE potentiated cell death in MCF-7 via apoptosis. Hence, further evaluation was carried out in order to elucidate the cytotoxic effect of ESEE towards this cell line. Many chemotherapeutics that provoke cytotoxicity in tumors seem to induce apoptosis as the common pathway of cell death (Hickman, 1992). In contrast to necrosis which is accompanied by passive cell swelling, injury of cytoplasmic organelles and rapid collapse of internal homeostasis (Bonfoco et al., 1995; Tan et al., 2009), the induction of apoptosis through activation by ischaemia and reperfusion of tissues is able to preserve cellular integrity of cells in the body (Kam and Ferch, 2000). Therefore, induction of cell death through apoptosis has

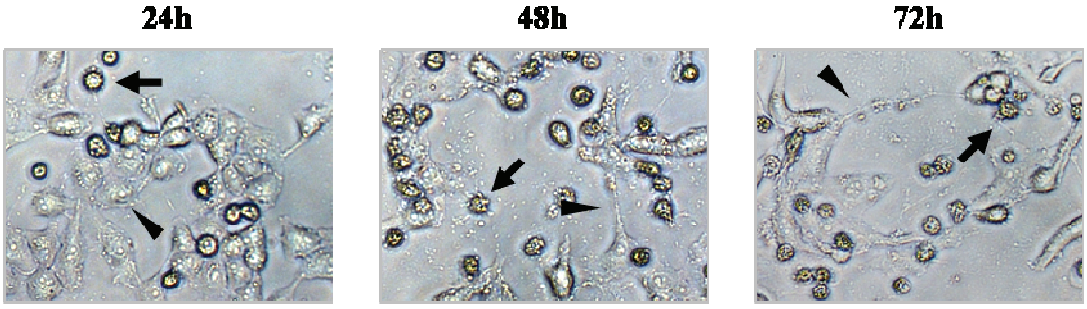

Figure 1. Cytotoxic effect of *E. scaber* towards MCF-7 was observable after treatment with 15 µg/mL of its ethanolic extract. Some of the cells detached from the culture plate while the rest remained attached showing some irregularity in cell morphology. Cell shrinkage, which is a sign of apoptosis, was observable in the treatment group. Formation of blebs on the cell membrane surface (indicated by arrow) and protrusion of microspikes from the cells (pointed by arrow head) agreed with the occurrence of apoptosis.

become a favorable therapeutic strategy for designing treatments against cancerous diseases (Alitheen et al., 2010, 2011). The occurrence of apoptosis is described as a cascade of programmed processes involving a series of characterized morphological and biochemical criteria (Majno and Joris, 1995; Sgonc and Gruber, 1998; Fadeel and Orrenius, 2005). Direct examination of the ESEE treated cells in culture plates (Figure 1) revealed characteristics such as intense surface zeiotic blebbing, protrusion of thin surface microspikes and loss of substratum adhesion from the cell culture plate, that were described by Collins et al. (1997) as signs of apoptosis. An anti-inflammatory study carried out by Ichikawa et al. (2006) concluded that isodeoxyelephantopin isolated from *E. scaber* is potential therapeutic agent for proinflammatory diseases by blocking of the NF-κB pathway. Also in their study, the compound was also identified to potentiate apoptotic effects through down-regulation of some NF-κB regulated gene products modulating cell proliferation, invasion and antiapoptosis. Therefore, the study was extended to investigate the occurrence of apoptosis in MCF-7 after ESEE treatment.

Cells treated with 15 µg/mL of ESEE after the respective periods were stained with AO and PI dyes to distinguish among viable, apoptotic and necrotic cells. Viable cells, with intact membranes, were stained green with AO and showed well-defined nuclear structures. Apoptotic cells, on the other hand, were also stained green with AO but exhibited chromatin condensation, nuclear genome fragmentation and membrane blebbing, which differentiated them from viable cells. The late apoptotic cells that possessed disintegrated membranes were additionally stained orange by PI dye but were differentiated from the morphological similar necrotic cells by the occurrence of fragmented DNA. As shown in Figure 2, these apoptotic events were increased significantly up to about 8 fold higher than control cells after 24 (52.72%) and 48 h (53.85%) of treatment with ESEE and the percentage was even higher after 72 h whereby 63.55% of apoptotic cells were detected. The percentage of necrotic cells observed was also increased but by a much lower extent from 4.50% at 24 h to 5.45 and 6.20% at 48 and 72 h, respectively.

Next, Tunel assay was carried out to confirm apoptosis in MCF-7 cells upon ESEE treatment. The method is based on the specific binding of terminal deoxynucleotidyl transferase (TdT) to incorporate biotinylated deoxyuridine to 3'-OH ends at sites of DNA breaks and accurate quantification of the incorporation signals was done using a flow cytometer. ESEE treatment was shown to mediate apoptosis through DNA fragmentation in MCF-7 cells (Figure 3). It is apparent that the untreated cells showed minute tunnel positive signals (less than 0.3%) at all three incubation periods. As opposed to this, treatment with ESEE significantly induced DNA fragmentation in the cells whereby 43.09% of the treated cells were TUNEL positive at 24 h and the rate increased to 95.23 and 96.94% after 48 and 72 h, respectively. A high percentage of DNA fragmentation at the late time points indicated that these cells were in the late stage of apoptosis after long exposure to ESEE and early apoptosis had take place at an earlier time point.

However, DNA fragmentation was shown to occur at a late stage during apoptosis (Collins et al., 1997). Hence, the cytotoxicity effect of ESEE on MCF cell line was also examined at earlier time frames (3, 6 and 12 h) to investigate the initiation of apoptotic event by using Annexin V/PI analysis, which serves as a useful specific marker for early phase apoptosis when the cell membrane is still intact (Koopman et al., 1994; Yeap et al., 2011). Assessment from the flow cytometric analysis showed increase of phosphatidylserine externalization in treated cells with time. As shown in Figure 4, early apoptotic signals could be traced in the cells as early as 3 h after incubation with 15 µg/mL of ESEE. Early apoptotic as well as late apoptotic and necrotic events were significantly increased when compared to untreated cells in the early time frames (3, 6 and 12 h). However, as the incubation period was increased, a shift from early apoptosis to late apoptosis was observable in the cells. In

Figure 2. Percentage of viable (V), apoptotic (A) and necrotic (N) cells in control and ESEE treated cells as determined by dual staining with acridine orange and propidium iodide. The cells were incubated for 24, 48 and 72h, respectively and the results are presented as means ± SEM of three independent experiments (*P < 0.05 for comparison of control cells to cells treated with ESEE).

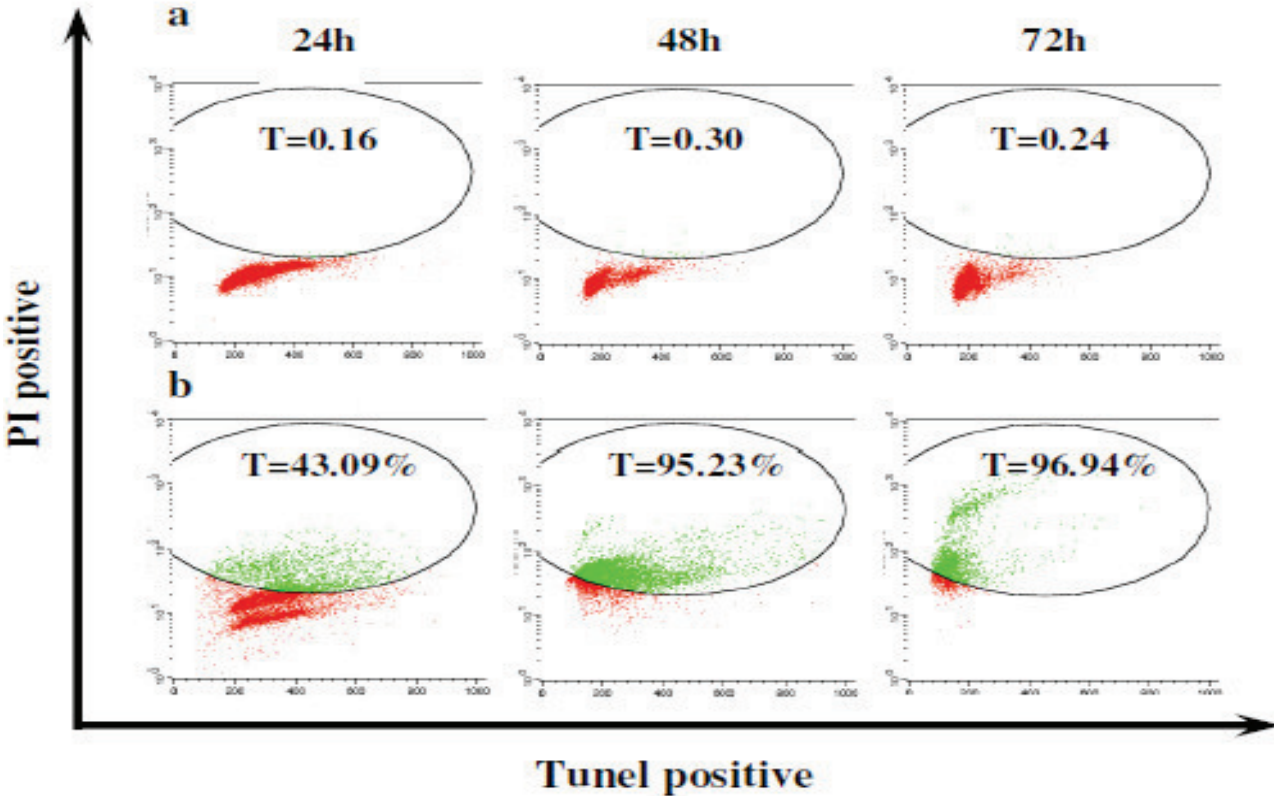

Figure 3. Effects of ESEE on DNA fragmentation. MCF-7 cells were cultured for 24, 48 and 72 h with or without the presence of 15 µg/mL of ESEE. Cells were then stained by TUNEL and analyzed by flow cytometry. Results are expressed as percentage of cells in the T (Tunel positive) region (*P < 0.05 for comparison of control cells to ESEE treated cells).

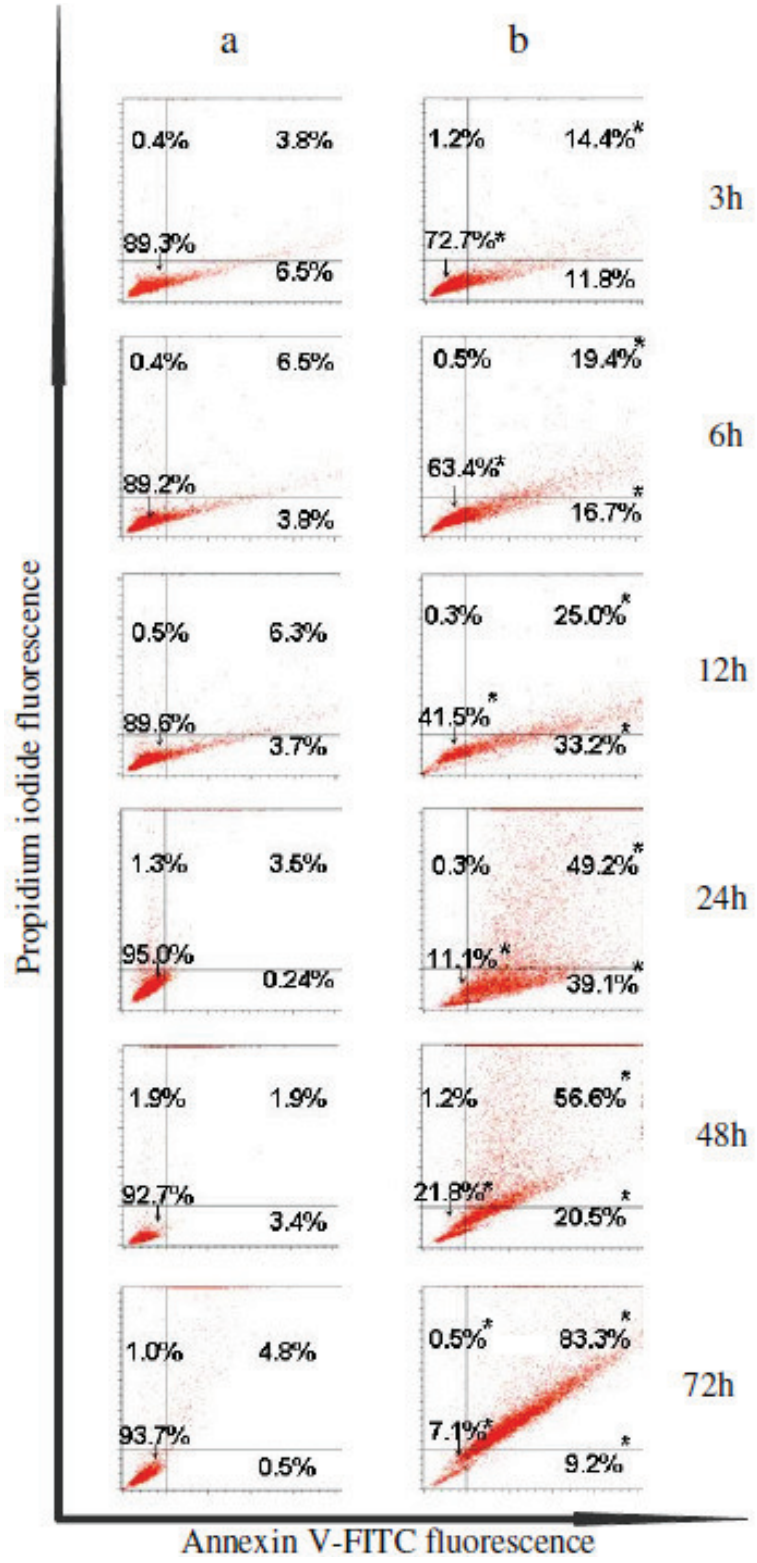

Figure 4. Determination of apoptotic events in MCF-7 cells treated with ESEE based on recognition of phosphatidylserine at the outer leaflet of cell membrane. (a) control group of untreated cells and (b) cells incubated with15 µg/mL of ESEE for respective periods as indicated on the right. The proportion of cells in each quadrant is represented as mean percentage in replicates of 3 independent experiments (*P < 0.05 for comparison of control cells to cells treated with ESEE).

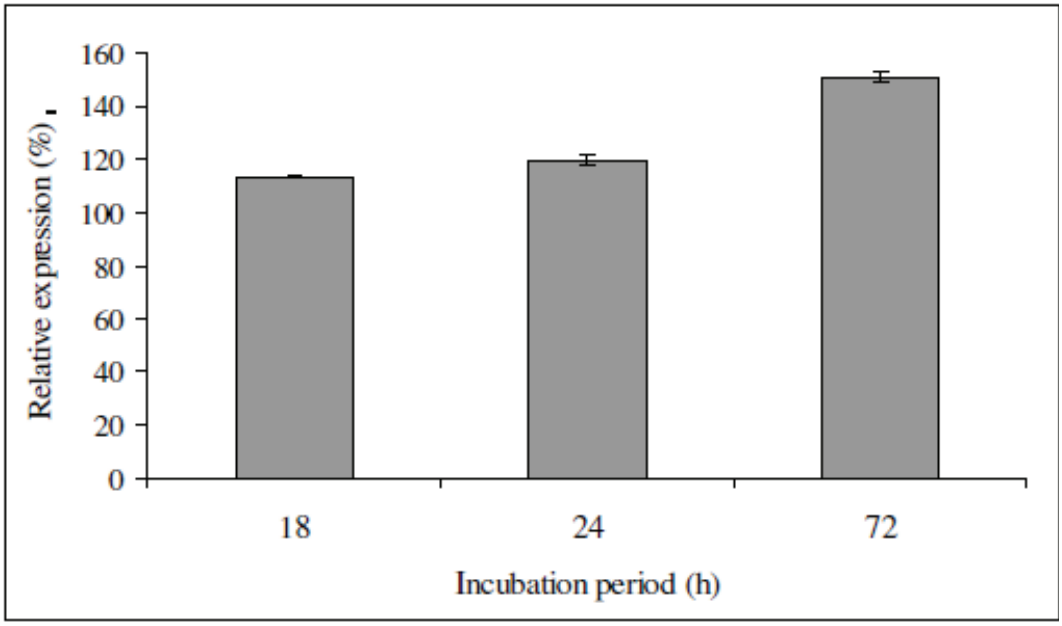

Figure 5. Relative expression of p53 protein in ESEE treated MCF-7 cells. The values are expressed as percentages of the untreated control group (expression of untreated control = 100%). Results are presented as means ± SEM of three independent experiments (*P < 0.05 for comparison of control cells to cells treated with ESEE).

comparison to increasing percentage of cells identified in both the early and late apoptotic quadrants from 3 to 12 h, early apoptotic signals decreased as ESEE incubation was prolonged from 24 to 72 h as opposed to the increasing percentage of cells detected in the late apoptosis quadrants.

It is convincing from the cytoxicity assays that ESEE induced apoptosis in the MCF-7 cell line in a time dependent and dosage dependent manner. We were then attempted to investigate further the alteration of p53 expression, a tumor suppressor protein, in this *in vitro* model for estrogen dependent human breast cancer. p53 protein acts as a key regulator modulating many downstream targets which play major roles in cellular processes such as programmed cell death and cell cycle control and thus serves as a critical target for development of anti-cancer agents (May and May, 1999). The expression of p53 was up-regulated for 13.35% after 18h as compared to the untreated control and was slightly elevated after 24 h of ESEE incubation (Figure 5). However, a marked increase of p53 expression (52.29%) was observed after 72 h of treatment, thereby showing that the MCF-7 cells could be stimulated to up-regulate p53 protein expression in response to ESEE treatment and this protein might play a role in leading to apoptotic cell death in the treated population. p53-dependent apoptosis can occur in response to many factors such as depletion of nucleotides and serum, DNA damage, hypoxia, radiation, activated oncogenes etc. (Linke et al., 1996; Hurd et al., 1997; May and May, 1999). It was shown that treatment with the antiestrogens tamoxifen, 4-hydroxytamoxifen (OHT) and ICI 164,384 in human breast cancer cells regulated the expression of p53 in breast cancer cells (Hurd et al., 1997) and the accumulation of p53 in breast cancer was correlated with estrogen receptors where p53 acted as a potent repressor for hormone-dependent transcriptional activity of the estrogen (Yu et al., 1997). The up-regulation of p53 expression by ESEE in this estrogen dependent human breast cancer cell line may be attributed to apoptosis induction via the estrogen dependent pathway. Furthermore, ESEE may also regulate cell cycle arrest in MCF-7 as p53 is well known for suppression of cell growth via G1 cell cycle arrest and negative regulatory effect on cell-cycle genes (Miller et al., 2005). However, further investigations are yet to be carried out to verify these hypotheses.

In conclusion, this study has proven that the ethanolic extract of *E. scaber* could inhibit growth and triggered time dependent and dosage dependent cell death in the MCF-7 breast cancer cell line via a p53 dependent apoptotic pathway. p53 appears to be a central protein modulating many critical pathways leading to development and design for anti-cancer agents. Hence, investigation on the downstream proteins associated with p53 would provide important clues for evaluating ESEE as a potential treatment for human breast cancer. In future studies, the effect of ESEE on estrogen receptor status and cell cycle r egulation may be investigated to explore the potency of ESEE in targeting breast cancers.

ACKNOWLEDGEMENTS

The authors would like to express gratitude to Prof. Tan Soon Guan for revising the manuscript as well as Mr. Lim Chung Lu and Mr. Chan for plant identification and herbarium deposition. This research was supported by the Research University Grant Scheme (RUGS) of Universiti Putra Malaysia, Malaysia.

REFERENCES

Alitheen NB, Ooon CL, Keong YS, Chuan TK, Li HK, Yong HW (2011). Cytotoxic effects of commercial wheatgrass and fiber towards human acute promyelocytic leukemia cells (HL60). Pak. J. Pharm. Sci., 24: 243-250.

Alitheen NB, Yeap SK, Shuhaimi M, Abdul MA, Mashitoh AR, Nordin L (2010). Cytotoxic Effect of Damnacanthal, Nordamnacanthal, Zerumbone and Betulinic Acid Isolated from Malaysian Plant Sources. I.F.R.J., 17: 711-719.

American Cancer Society (2007). Breast cancer facts and figures 2007-2008. American Cancer Society.

Balachandran P, Govindarajan R (2005). Cancer-an ayurvedic perspective. Pharmacol. Res., 51: 19-30.

Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA (1995). Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Proc. Natl. Acad. Sci. U. S. A., 92: 7162-7166.

Chiaramonte D, Lao L (2010). Integrating Chinese and Western medicine in cancer treatment. In: Support. Cancer Care Chin. Med., 341-361.

Collins JA, Schandl CA, Young KK, Vesely J, Willingham MC (1997). Major DNA fragmentation is a late event in apoptosis. J. Histochem. Cytochem., 45: 923-934.

Collins K, Jacks T, Pavletich NP (1997). The cell cycle and cancer. Proc. Natl. Acad. Sci. U. S. A., 94: 2776-2778.

Daisy P, Rayan NA, Rajathi D (2007). Hypoglycemic and other related effects of *Elephantopus scaber* extracts on alloxan induced diabetic rats. J. Biol. Sci., 7: 433-437.

Ernst E (2000). Prevalence of use of complementary/alternative medicine: a systematic review. W. H.O., 78: 252-257.

Fadeel B, Orrenius S (2005). Apoptosis: a basic biological phenomenon with wide-ranging implications in human disease. J. Int. Med., 258: 479-517.

Fisher B, Costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux A, Bevers TB, Kavanah MT, Atkins JN, Margolese RG, Runowicz CD, James JM, Ford LG, Wolmark N (2005). Tamoxifen for the prevention of breast cancer: Current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. J. Natl. Cancer Inst., 97: 1652-1662.

Gurib-Fakim A, Sewraj M, Gueho J, Dulloo E (1993). Medicalethnobotany of some weeds of Mauritius and Rodrigues. J. Ethnopharmacol., 39: 175-185.

Hickman JA (1992). Apoptosis induced by anticancer drugs. Cancer Metastasis Rev., 11: 121-139.

Ho WY, Ky H, Yeap SK, Rahim RA, Omar AR, Ho CL, Alitheen NB (2009). Traditional practice, bioactivities and commercialization potential of *Elephantopus scaber* Linn. J. Med. Plants Res., 3: 1212-1221.

Hurd C, Khattree N, Dinda S, Alban P, Moudgil VK (1997). Regulation of tumor suppressor proteins, p53 and retinoblastoma, by estrogen and antiestrogens in breast cancer cells. Oncogene, 15: 991 - 995.

Ichikawa H, Nair MS, Takada Y, Sheeja DBA, Kumar MAS, Oommen OV, Aggarwal BB (2006). Isodeoxyelephantopin, a novel sesquiterpene lactone, potentiates apoptosis, inhibits invasion, and abolishes osteoclastogenesis through suppression of nuclear factor-κB (NF-κB) activation and NF-κB-regulated gene expression. Clin. Cancer Res., 12: 5910-5918.

Inta A, Shengji P, Balslev H, Wangpakapattanawong P, Trisonthi C (2008). A comparative study on medicinal plants used in Akha's traditional medicine in China and Thailand, cultural coherence or ecological divergence? . J. Ethnopharmacol., 116: 508-517.

Kam PC, Ferch NI (2000). Apoptosis: mechanisms and clinical implications. Anaesthesia, 55(11): 1081-1093.

Koopman G, Reutelingsperger C, Kuijten G, Keehnen R, Pals S, Van OM (1994). Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood, 84: 1415-1420.

Lee S, Xiao C, Pei S (2008). Ethnobotanical survey of medicinal plants at periodic markets of Honghe Prefecture in Yunnan Province, SW China. J. Ethnopharmacol., 117: 362-377.

Liang Q, Min Z, Tang Y (2008). A new elemanolide sesquiterpene lactone from *Elephantopus scaber*. J. Asian Nat. Prod. Res., 10: 403-407.

Liang QL, Min ZD (2002). Sesquiterpene lactones from *Elephantopus scaber*. Chin. J. Chem., 13: 343-344.

Linke SP, Clarkin KC, Leonardo AD, Tsou A, Wah GM (1996). A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Genes Dev., 10: 934-947.

Majno G, Joris I (1995). Apoptosis, oncosis, and necrosis. An overview of cell death. Am. J. Pathol., 146: 3-15.

Mann J (2000). Natural products in cancer chemotherapy: past, present and future. Cancer, 2: 143-148.

May P, May E (1999). Twenty years of p53 research: structural and functional aspects of the p53. Protein Oncogene, 18: 7621 - 7636.

Miller L, Smeds J, George J, Vinsensius B, Vega, Vergara L, Ploner A, Pawitan Y, Hall P, Klaar S, Liu E, Bergh J (2005). An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. Proc. Natl. Acad. Sci. U. S. A., 102(38): 13550-13555.

Molassiotis A, Fernadez-Ortega P, Pud G, Ozden D, Scott J, Panteli V, Margulies A, Browall M, Magri M, Selvekerova S, Madsen E, Milovics L, Bruyns I, Gudmundsdottir G, Hummerston S, Ahmad A, Platin N, Kearney N, Patiraki E (2005). Use of complementary and alternative medicine in cancer patients: a European survey. Ann. Oncol., 16: 655-663.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63.

Ong H, Nordiana M (1999). Malay ethno-medico botany in Machang, Kelantan, Malaysia. Fitoterapia, 70: 502-513.

Sgonc R, Gruber J (1998). Apoptosis detection: an overview. Exp. Gerontol., 33: 525-533.

Shelley B (2009). Ethnobotany and the process of drug discovery: A laboratory exercise. Am. Biol. Teach., 71: 541-547.

Tan BK, Alitheen NB, Yeap SK, Ali AM, Mawardi R (2009). Cytotoxic effect of 2',3'-epoxy isocapnolactone and 8-hydroxyisocapnolactone-2'3'-diol isolated from *Micromelum minutum* (G.Forst.) Wight & Arn. in human T-lymphocyte leukemia CEM-SS cells. Afr. J. Biotechnol., 8: 4632-4641.

Than NN, Fotso S, Sevvana M, Sheldrick G, Fiebig H, Kelter G, Laatsch H (2005). Sesquiterpene lactones from *Elephantopus scaber*. Z. Naturforsch., 60: 200-204.

Veronesi U, Misonneuve P, Rotmensz N, Bonanni B, Boyle P, Viale G, Costa A, Sacchini V, Travaglini R, D'Aiuto G, Oliviero P, Lovison F, Gucciardo G, Del TMR, Muraca MG, Pizzichetta MA, Conforti S, Decensi A (2007). Tamoxifen for the prevention of breast cancer: late results of the Italian Randomized Tamoxifen Prevention Trial among women with hysterectomy. J. Natl. Cancer Inst., 99: 727-737.

World Health Organization, (2011). Cancer. Fact sheet No. 297. Geneva: World Health Organization.

Xu G, Liang Q, Gong Z, Yu W, He S, Xi L, (2006). Antitumor activities of the four sesquiterpene lactones from *Elephantopus scaber* L. Exp. Oncol., 28: 106-109.

Yeap SK, Ho WY, Beh BK, Liang WS, Ky H, Yousr AHN, Alitheen NB (2010). *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. J. Med. Plants Res., 4: 2787-2812.

Yeap SK, Alitheen NBM, Ho WY, Omar AR, Ali AM, Beh BK, Yousr

AHN (2011). Immunomodulatory role of *Rhaphidophora korthalsii* methanol extract on human peripheral blood mononuclear cell proliferation, cytokine secretion and cytolytic activity. J. Med. Plants Res., 5: 958-965.

Yu CL, Driggers P, Barrera-Hernandez G, Nunez SB, Segars JH, Cheng SY (1997). The Tumor Suppressor p53 is a negative regulator of estrogen receptor signaling pathways. Biochem. Biophys. Res. Commun., 239: 617-620.