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Full Length Research Paper

Antiproliferative and apoptotic effect of *Astrodaucus orientalis* (L.) drude on T47D human breast cancer cell line: Potential mechanisms of action

M. H. Abdolmohammadi[1,5*], S. Fouladdel[1,2], A. Shafiee[3], G. Amin[4], S. M. Ghaffari[5] and E. Azizi[1,2]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[2]National Center of Excellence of Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[3]Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[4]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[5]Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran.

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Breast cancer is one of the most common cancers in women. Here, we report the antiproliferative and the mechanism of cell death exhibited by *Astrodaucus orientalis* in human breast carcinoma cell line, T47D. The antiproliferative effect determined with MTT assay and the changes in cell cycle pattern were studied with DAPI reagent and Annexin V/PI method used for apoptotic analysis. The p53 and Bcl-2 are believed to play a crucial role in tumorigenesis and cell death. The p53 and Bcl-2 genes and proteins expression alterations in T47D were studied using RT-PCR and immunocytochemistry technique. Both extracts showed strong antiproliferative effects on cells. Root extract showed relatively similar cell cycle pattern to RPMI but the aerial extract showed cell cycle arrest in G2/M. Both extracts induced apoptosis and the effect of aerial extract was more prominent than that of root extract. The p53 gene expression decreased in presence of extracts and Bcl-2 gene expression also significantly decreased as their expressions had not detectable. Both extracts also showed decrease in p53 and Bcl-2 protein expression. These data are first reported on potential of anticancer activity of *A. orientalis* extracts and one of its possible mechanisms of action on cancer cell proliferation.

Key words: ***Astrodaucus orientalis***, T47D cells, breast cancer, p53, Bcl-2, flowcytometry, RT-PCR, immunocytochemistry.

INTRODUCTION

Worldwide, one million women are estimated to be newly diagnosed with breast cancer each year (Hanf and Gonder, 2005). It is both genetically and histopathologically heterogeneous, and the mechanisms underlying breast cancer development remains largely unknown (Hedenfalk et al., 2002). The development of breast cancer involves several types of genes that need to be activated or inactivated in order to promote malignancy (Ingrasson, 2001). Cancer chemoprevention applies specific natural or synthetic chemical compounds to inhibit or reverse carcinogenesis and to suppress the development of cancer from premalignant lesions (Sarkar and Li, 2007). A major problem with present cancer chemotherapy is the serious deficiency of active drugs for the curative therapy of tumors (Valeriote et al., 2002; Kinghorn et al., 2003). For thousands of years, natural

*Corresponding author. E-mail: mh_abdolmohammadi@ibb.ut.ac.ir Tel: +9821-66409517. Fax: +9821-66404680.

products have played an important role throughout the world in treatment and prevention of human diseases (Kwon et al., 2007; Chin et al., 2006). Over 60% of currently used anticancer agents are derived in one way or another from natural sources (Balunas and Kinghorn, 2005; Cragg and David, 2005). The search for anticancer agents from plant sources started in the 1950s by discovery and development of the vinca alkaloids, vincristine, and the isolation of the cytotoxic podophyllotoxins (Tsuda et al., 2004; Srivastava et al., 2005). On the basis of the mechanism through which they exert anticancer effects, chemopreventive agents can be divided into two groups: antimutagenic and antiproliferative; antimutagens reduce formation of mutagens or carcinogens thereby preventing DNA damage. Alternatively, chemopreventive agents may exert antiproliferative effects via induction of cell cycle arrest or apoptosis, inhibition of angiogenesis, induction of terminal differentiation, and inhibition of ontogeny activity or DNA synthesis. In particular, apoptosis, a physiological model of cell death, in which the cell itself executes the program for its own demise and subsequent removal, is an active field of research worldwide by scientists engaged in the search for cancer chemopreventive agents (Wu et al., 2005; Schmitt, 2007). Among chemotherapeutic drugs, the agents that alter the cell cycle have been of particular interest, since cell cycle regulation is a basic mechanism underlying cell fate, that is, proliferation, differentiation or death (Dobashi et al., 2003). Thus uncontrolled cell proliferation is one of the main hallmarks of cancer, and tumor cells acquire damage in genes that are directly involved in regulation of the cell cycle (Tachibana et al., 2005; Sandal, 2002). Most, if not all, human cancers show a deregulated control of G1 phase progression, a period when cells decide whether to start proliferation or to stay quiescent (Golias et al., 2004; Hung et al., 1996). Tumorigenesis is thought to result from a series of progressive gene alterations, including activation of ontogene and inactivation of tumor suppressor genes. During the following years it became evident that wild-type p53 act as a tumor suppressor and that inactive p53 is responsible for the damaging effects such as cancers (Romer et al., 2006; Niida and Nakanishi, 2006). The p53 is found mutated in all of the major histogenetic groups, including cancers of the colon, stomach, breast, ovary, lung, brain, and esophagus. It is estimated that p53 mutations are the most frequent genetic events in human cancers and account for more that 50% of all cases (Feki and Irminger-Finger, 2004; Michalak et al., 2005). Bcl-2 is the founding member of a growing family whose members have emerged as important regulators of cell death. The Bcl-2 family members can have anti- and pro-apoptotic functions (Youle and Strasser, 2008; Willis et al., 2003). Bcl-2 proteins are involved in the control of apoptosis upstream of caspase-3 activation and of any other irreversible cellular damage; they might be impor- tant in deciding whether a cell will live or die (Burlacu, 2003). One of the first death regulating genes to be identified was Bcl-2, an anti-apoptotic gene. Bcl-2 can prevent apoptosis induction by a wide variety of stimuli. Expression of the Bcl-2 gene is regulated by estrogens in mammary epithelial cells and estrogen receptor positive breast cancer cell line. Expression of the Bcl-2 protein has been detected by immunochemical methods in nearly 80% of breast cancers derived from women with primary tumor and having either node positivity or negativity. Thus expression of Bcl-2 is commonly associated with favorable prognosis in breast cancer (Krajewski et al., 1999; Daidone et al., 1999).

One of the approaches used in drug discovery, is the ethnomedical data approach, in which the selection of a plant is based on the prior information on the use of the plant in the folk medicine. It is generally known that ethnomedical data provides substantially increased chance of finding active plants relative to random approach (Lee, 1999; Montbriand, 2004). Thus ***A. orientalis*** (L) Drude, a plant of the family of ***Umbelliferae*** used as a remedy in cancer related diseases was evaluated for its properties. In Iran, the genus ***Astrodaucus*** is represented by two species, ***A. persicus*** (Boiss.) Drude and ***A. orientalis*** (L.) Drude which grow wild in different regions of Iran and nearby countries, such as central and southern Russia, northern and north-western Caucasus, the western desert of Syria, inner Anatolia and central Asia. In Iran, ***A. orientalis*** is mainly distributed in Azarbayejan, Mazandaran, Khorasan, Ghazvin provinces. The chemical composition of the essential oils of ***A. orientalis*** leaves and seeds were investigated but cytotoxicity and anticancer activities of this plant have not been reported previously (Mirza et al., 2003). Therefore, the main aim of this study was to evaluate the cytotoxic properties and antiproliferative effect of ***A. orientalis*** extracts and to determine the possible mechanisms of cell cycle alterations and induction of apoptosis elicited by the extracts on breast cancer cells.

MATERIALS AND METHODS

Plant material

Astrodaucus orientalis plant (Voucher No. 6643-TEH) was collected from between Mianeh and Gharachaman in Iran and was identified by Dr. G. R. Amin. The aerial part and root were isolated, kept under shade till drying and chopped finely using a blender. The plant powders were stored at -20^{o}C.

Preparation of extracts

One hundred grams of dried material of aerial part or root were extracted with methanol by soxhlet extraction. The methanolic extracts were filtered and evaporated to dryness under reduced pressure in a rotatory evaporator. The resulting residues for each part of plant were stored at -20^{o}C. The extract was dissolved in DMSO (Sigma), sterilized by filtration and subsequently diluted to appropriate working concentrations with RPMI culture medium (Tan et al., 2005).

Cell line and culture medium conditions

The human breast cancer T47D cell line (ATCC HTB-133, USA) was obtained from the cell bank of Pasteur Institute in Tehran (IRAN). This hormone sensitive breast cancer cell line was grown routinely as monolayer culture in RPMI-1640 culture medium supplemented with 100 U/mL of penicillin, 100 µg/mL of streptomycin and 10% heat inactivated FBS at 37°C in 5% CO_2 incubator (Tan et al., 2005).

In vitro cytotoxicity assay

Cells were used in cytotoxicity studies when 90% confluence was reached in T25 flasks. Cells were harvested with trypsin/EDTA, washed with PBS and counted using trypan blue dye exclusion method. T47D cells were seeded into 96 well plates at a density of 10^4 cells/well and left to attach to the plates for 48 h. After 48 h, cells were incubated for 2, 4 and 6 days with various concentrations of extracts. After the exposure time, the cells were incubated with 25 µL of MTT (4 mg/ml) at 37°C for 3 h. After dissolving the formazan crystals in DMSO, plates were read in a microplate reader (SUNRISE TECAN, Austria) at 540 nm against 620 nm. This experiment was performed in triplicates and repeated 3 times. Mean values±SE for each concentration was determined (Mosmann, 1983).

Analysis of cell cycle

Cell cycle phase distribution was determined by analytical DNA flowcytometry. T47D cells were incubated for 48 h with IC_{50} concentration of aerial and root part extracts of ***A. orientalis***. Extracts, TAM 10 µM and control RPMI treated T47D cells were harvested and adjusted to 5×10^5 cells/ml and stained with DAPI reagent for 30 min at 4°C in dark. The PARTEC flowcytometer with FloMax software was used to analysis DNA content using UV light at FL4. The percentage of cells in the various phases was determined and statistical analysis of data from flow cytomtric experiments was carried out (Dobashi et al., 2003).

Analysis of apoptosis

Five hundred thousand cells per well were plated in six well plates for 48 h to allow cells to attach and treated with controls (RPMI and TAM 10 µM) with plant extracts at IC_{50} concentrations. After 48 h of treatment, both adherent and floating cells were harvested and washed with PBS and then incubated with Annexin V-FITC and PI (APOPTEST^{TM}-FITC kit, NEXINS RESEARCH, Netherlands) as described by the manufacturer. Data on 10,000 cells were acquired and processed using a PARTEC flowcytometer with FloMax software.

RNA isolation

The mRNA expression levels of p53 and Bcl-2 carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, the T47D cells were cultured in T25 flasks and maintained in RPMI medium for 48 h. The RPMI medium was supplemented with FBS and pen-strep. Concentration of aerial part and root of ***A. orientalis*** methanolic extracts needed to achieve 50% growth inhibition was added to flask and incubated for 48 h. Total cellular RNA was isolated from the untreated (control flask) and treated cells using TriPure isolation reagent (Roche, Germany) according to manufacturer's protocol.

RT-PCR

cDNA was synthesized from 2 µg of total isolated RNA by incubation for 1 h at 42ºC with M-MLV reverse transcriptase (Fermentas) and oligo $(dT)_{18}$ primer according to the manufacturer's instruction. Then 2.5 µl of the reaction mixture was subjected to polymerase chain reaction (PCR) to amplify sequence of p53 and Bcl-2 using specific primers (p53 Forward: 5′-CTGAGGTTGGCTCTGACTGTACCACCATCC-3′; p53 reverse: 5′-CTCATTCAGCTCTCGGAACATCTCGAAGCG-3′ and Bcl-2 Forward: 5′-GTTCGGTGGGGTCATGTGTGTGGAGA-3′; Bcl-2 Reverse: 5′-GCTGATTCGACGTTTTGCCTGAAGAC-3′). As an internal control, the house keeping gene β-Actin (β-Actin Forward: 5′-ACGGGGTCACCCACACTGTGC-3′; β-Actin Reverse: 5′-CTAGAAGCATTTGCGGTGGACGATG-3′) was co-amplified in each reaction. The PCR reactions was carried out in a final volume of 50 µl containing 1x PCR buffer and 5U/µl Taq-polymerase (Fermentas), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.4 µM of each primer. The template was denatured for 5 min at 94ºC, followed by amplification cycles at 94ºC for 1 min, 69ºC (for p53, 64ºC for Bcl-2 and 57ºC for β-actin) for 1min and 72ºC for 1.20 min, and terminated with an additional extension step for 8 min at 72ºC. The PCR products were visualized using 1.2% agarose gel electrophoresis with ethidium bromide staining. In negative control, template cDNA was replaced by 1% DEPC-water.

Immunocytochemical assay of p53 and Bcl-2 proteins

The T47D cells were seeded in 8 well chamber slides (Lab Teck, USA) in RPMI 1640 culture medium and incubated in humidified CO_2 incubator (5% CO_2, 37°C). After 48 h TAM 10 µM was added to pairs of wells and the IC_{50} concentration of extracts of aerial part and root of ***A. orientalis*** were added to another pairs of wells. The medium of the control wells remained RPMI 1640. After 48 h incubation at 37°C these wells were suctioned and cells were washed with cold PBS twice and fixed in methanol: acetone (9:1) for 30 min at -20°C. Endogenous peroxidase activity and non-specific binding sites were blocked by incubating fixed cells in 3% H_2O_2 in methanol for 30 min followed by Ultra V block (Labvision, USA) for 10 min. For immunocytochemical assay the cells were incubated overnight with primary monoclonal antibody against p53 and Bcl-2 at dilution of 1:75 at 4°C. The results were visualized using the streptavidine-biotin immunoperoxidase detection kit and AEC chromogen (Labvision, USA) based on the manufacturer's instruction with necessary modifications. Finally, cells were counter-stained with Meyer's hematoxyline (DakoCytomation, Denmark), mounted and studied under light microscope. A section in which incubation with the primary antibody was omitted used as negative control.

Statistical analysis

Data are reported as means±SE, and comparisons were analyzed by one-way analysis of variance (ANOVA) with a Tukey post hoc test to identify between group differences ($P < 0.05$) using SPSS software (version 11.5).

RESULTS

Cytotoxicity activity of the extracts of *A. orientalis* on T47D cells

The antiproliferative effects of plant extracts in comparison to TAM 10 µM on T47D cells was determined using MTT method. As shown in Figures 1 and 2 the results demonstrated that the ***in vitro*** screening of the extracts of

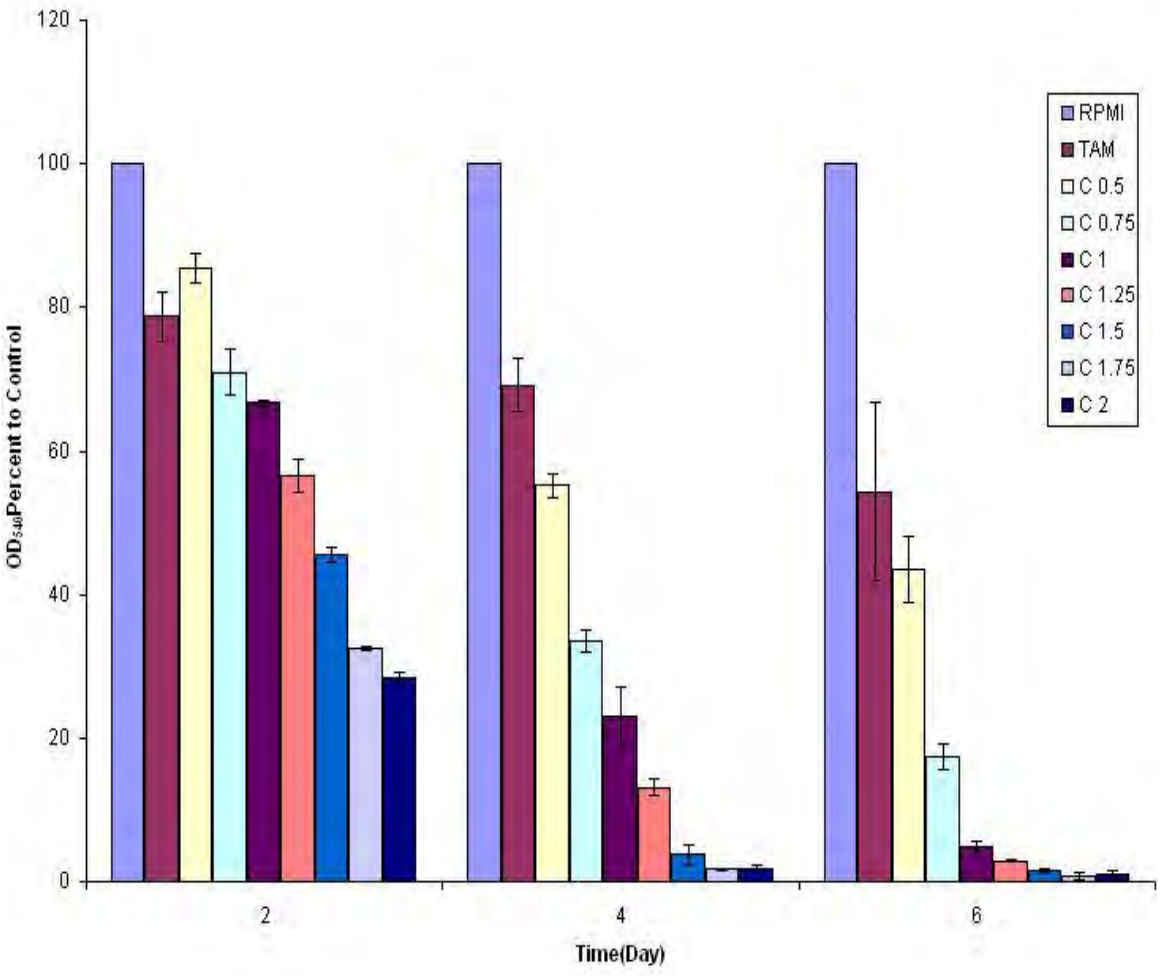

Figure 1. Cytotoxicity of different concentrations of aerial part extracts of ***A. orientalis*** on T47D cells. Cells were seeded into 96-well plates (10^4 cell/well). Two days later, the cells were incubated with RPMI as negative control and Tamoxifen 10µM as positive control and different concentrations of extract of ***A. orientalis*** aerial part (C) for 2, 4 and 6 days. Cell proliferation was determined by MTT assay. The data represents the mean±SE of 3 independents each in triplicate format.

aerial part and root of ***A. orientalis*** on breast carcinoma T47D cell line produced a time and dose dependent manner inhibition on cell proliferation. Based on these results the IC_{50} of aerial extract 1.5 mg/ml and root extract 2 mg/ml were calculated.

Cell cycle analysis

In order to determine the cell cycle pattern of T47D cells, the DNA content of RPMI, Tamoxifen and ***A. orientalis*** extracts treated cells were analyzed following DAPI staining and flowcytometric method. As shown in Figures 3 and 4 the cell cycle phase distribution was quantified from 3 independent sets of measurements that showed relatively similar pattern to RPMI which was completely different than Tamoxifen but the aerial extract of plant showed significantly cell cycle arrest in G2/M.

Apoptosis analysis

Apoptosis confirmation was carried out using the APOPTEST^{TM}-FITC kit (NEXINS RESEARCH, Netherlands). This test revealed that the negatively charged phospholipids phosphatidylserine found on the interior surface of the plasma membrane of the cells is translocated to the cell surface during apoptosis. Figure 5 present the effect of 48 h of incubation with plant extracts versus RPMI and TAM 10µM controls on the T47D cells. Both extracts induced apoptosis after 48 h incubation. In addition, the effect of aerial extract was more prominent than that of root extract.

Expression level of p53 and Bcl-2 genes

In order to determine the expression level of cancer rela-

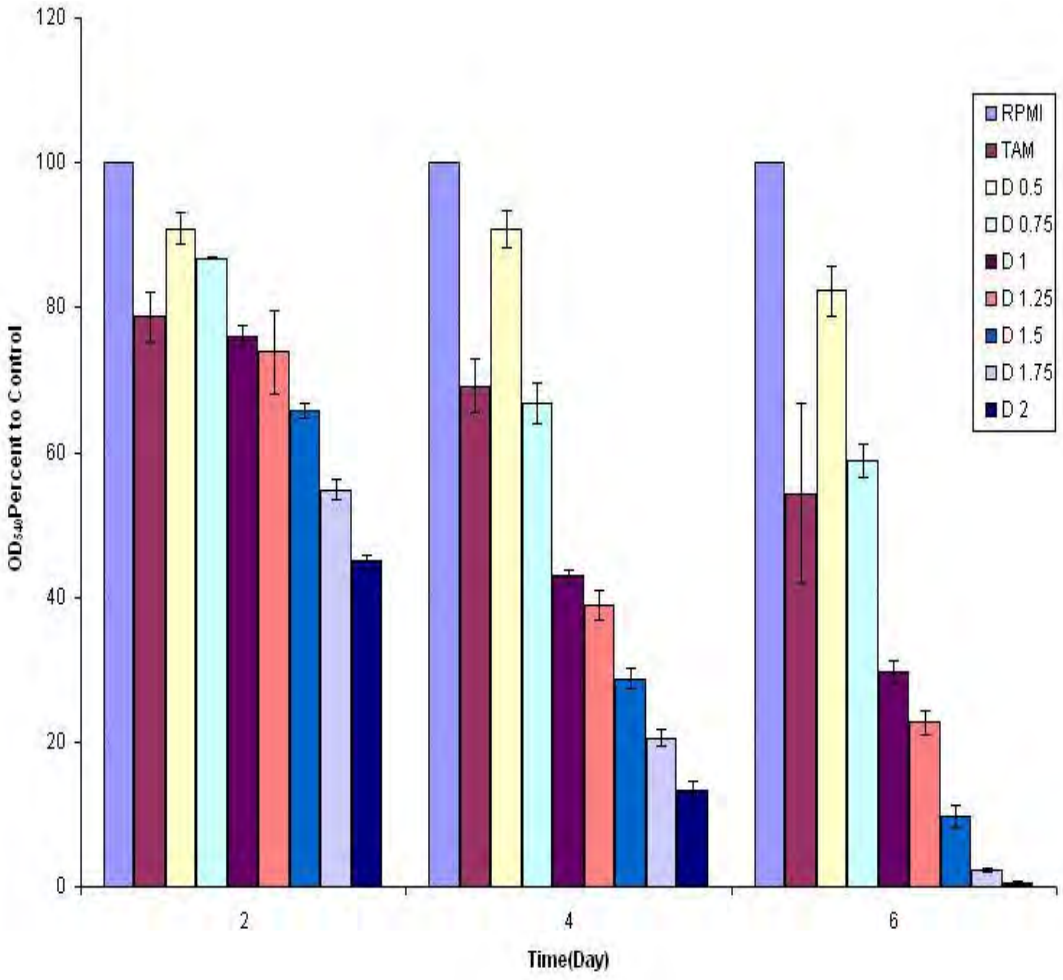

Figure 2. Cytotoxicity of different concentrations of root extracts of ***A. orientalis*** on T47D cells. Cells were seeded into 96-well plates (10^4 cell/well). Two days later, the cells were incubated with RPMI as negative control and Tamoxifen 10µM as positive control and different concentrations of extracts of ***A. orientalis*** root part (D) for 2, 4 and 6 days. Cell proliferation was determined by MTT assay. The data represents the mean±SE of 3 independents each in triplicate format.

ted genes induced in the ***A. orientalis*** aerial part and root methanolic extracts treated T47D cells, the mRNA levels of p53 and Bcl-2 were evaluated by RT-PCR. Quantification of each band was performed by densitometry analysis software and results expressed as the ratio (p53/β-actin or Bcl-2/β-actin) in percent to RPMI. Mean differences with $p<0.05$ were considered statistically significant. As shown in Figure 6 in comparison to RPMI control, the p53 gene expression decreased in presence of extracts and Bcl-2 gene expression also significantly decreased in presence of aerial and root extracts as their expressions had not been detectable.

Immunocytochemistry of p53 and Bcl-2

For immunocytochemical investigation of p53 and Bcl-2 expression in T47D cells treated with methanolic extracts of aerial part and root of ***A. orientalis*** the cells were immunostained with primary antibody for p53 and Bcl-2 as described in the methods. In the presence of plant extracts nuclear staining of p53 and cytoplasmic staining of Bcl-2 were decreased in T47D cells in compare to RPMI control as showed in Figures 7 and 8.

DISCUSSION

In this study the antiproliferative effects of plant extracts on T47D cells was determined using MTT methods. Results demonstrated that the extracts produced a time and dose dependent manner inhibition on cell proliferation. Based on these results the IC_{50} of aerial extract 1.5 mg/ml (C, 1.5 mg/ml) and root extract 2 mg/ml (D, 2 mg/ml) were measured. In order to determine the cell cycle pattern, the DNA content of RPMI, Tamoxifen

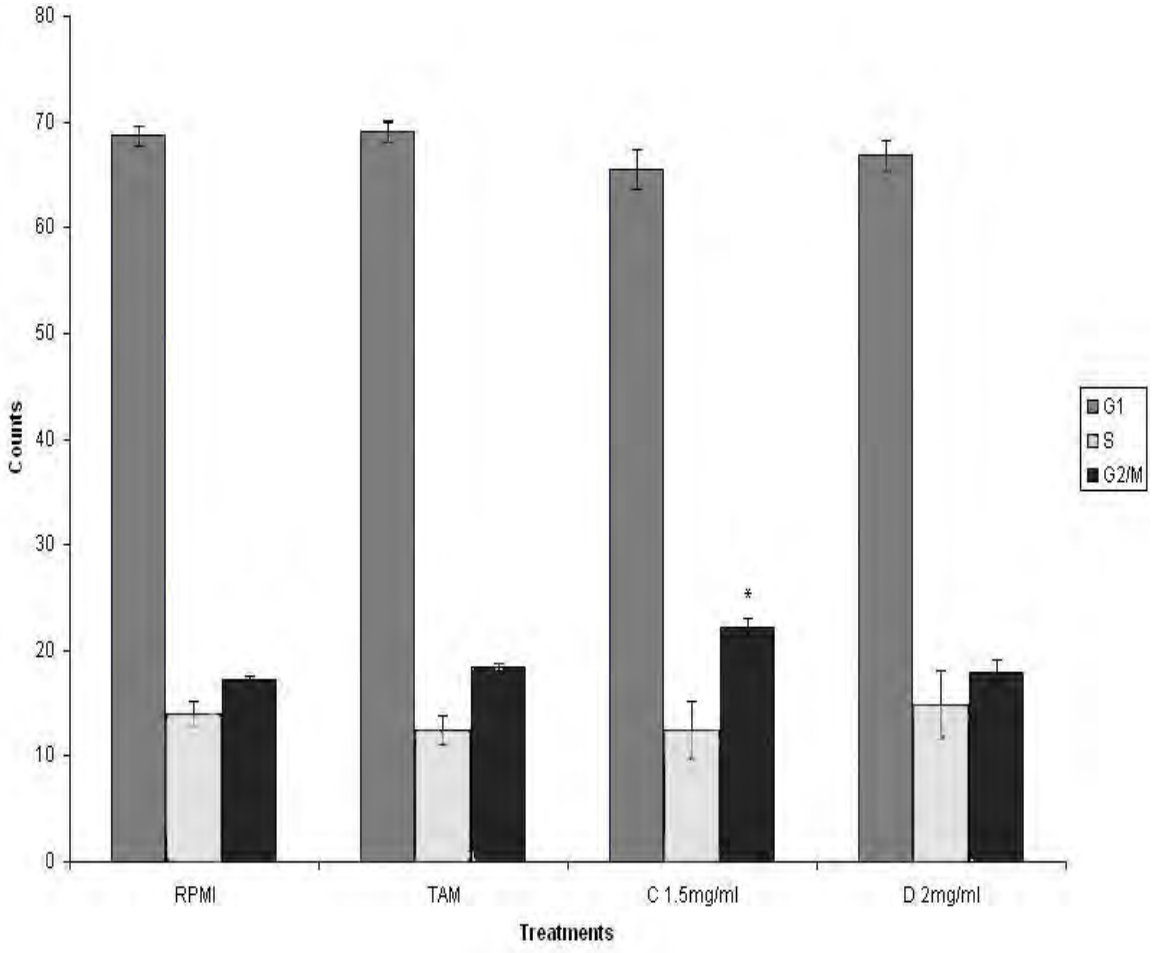

Figure 3. Cell cycle phases distribution of T47D cells. Cells (5×10^5 cells/ml) treated with aerial (C 1.5mg/ml) and root (D 2mg/ml) as well as RPMI or Tamoxifen 10µM were prepared for cell cycle analysis after 48 hours exposure. Cell stained with DAPI reagent were analyzed with PARTEC flowcytometer equipped with UV light at FL4. The data represent the mean±SE of 3 independent experiments. * mean differences with $p< 0.05$ were considered statistically significant.

and ***A. orientalis*** extracts treated cells were analyzed following DAPI staining and flowcytometric method. Apoptosis confirmation was carried out using the Annexin V/PI method. Simultaneous staining of cells with FITC-Annexin V (green fluorescence) and the non-vital dye propidium iodide (red fluorescence) allows the discrimination of intact cells (FITC−PI−), early apoptotic (FITC + PI−), and late apoptotic or necrotic cells (FITC + PI +) via bivariate analysis (Fieder et al., 2005). Both extracts induced apoptosis after 48 h incubation. In addition, the effect of aerial extract was more prominent than that of root extract. In order to determine the expression level of cancer related genes induced in the ***A. orientalis*** aerial part and root methanolic extracts treated T47D cells, the mRNA levels of p53 and Bcl-2 were evaluated by RT-PCR. In comparison to RPMI control, the p53 gene expression decreased in presence of extracts and Bcl-2 gene expression also significantly decreased in presence of aerial and root extracts as their expressions had not been detectable. For immunocyto-chemical investigation of p53 and Bcl-2 expression in T47D cells treated with methanolic extracts of aerial part and root of ***A. orientalis*** the cells were immunostained with primary antibody for p53 and Bcl-2 as described in the methods. In the presence of plant extracts nuclear staining of p53 and cytoplasmic staining of Bcl-2 were decreased in T47D cells in compare to RPMI control.

Chemopreventive agents comprise diverse groups of compounds with different mechanisms of action with ultimate ability to induce apoptosis. Understanding the modes of action of these compounds should provide useful information for their possible application in cancer prevention and perhaps also in cancer therapy (David, 2004). Cell cycle modulation by various natural and synthetic agents is gaining widespread attention in recent years. Given that disruption of cell cycle plays a crucial role in cancer progression, its modulation by phyto-chemicals seems to be a logical approach in control of

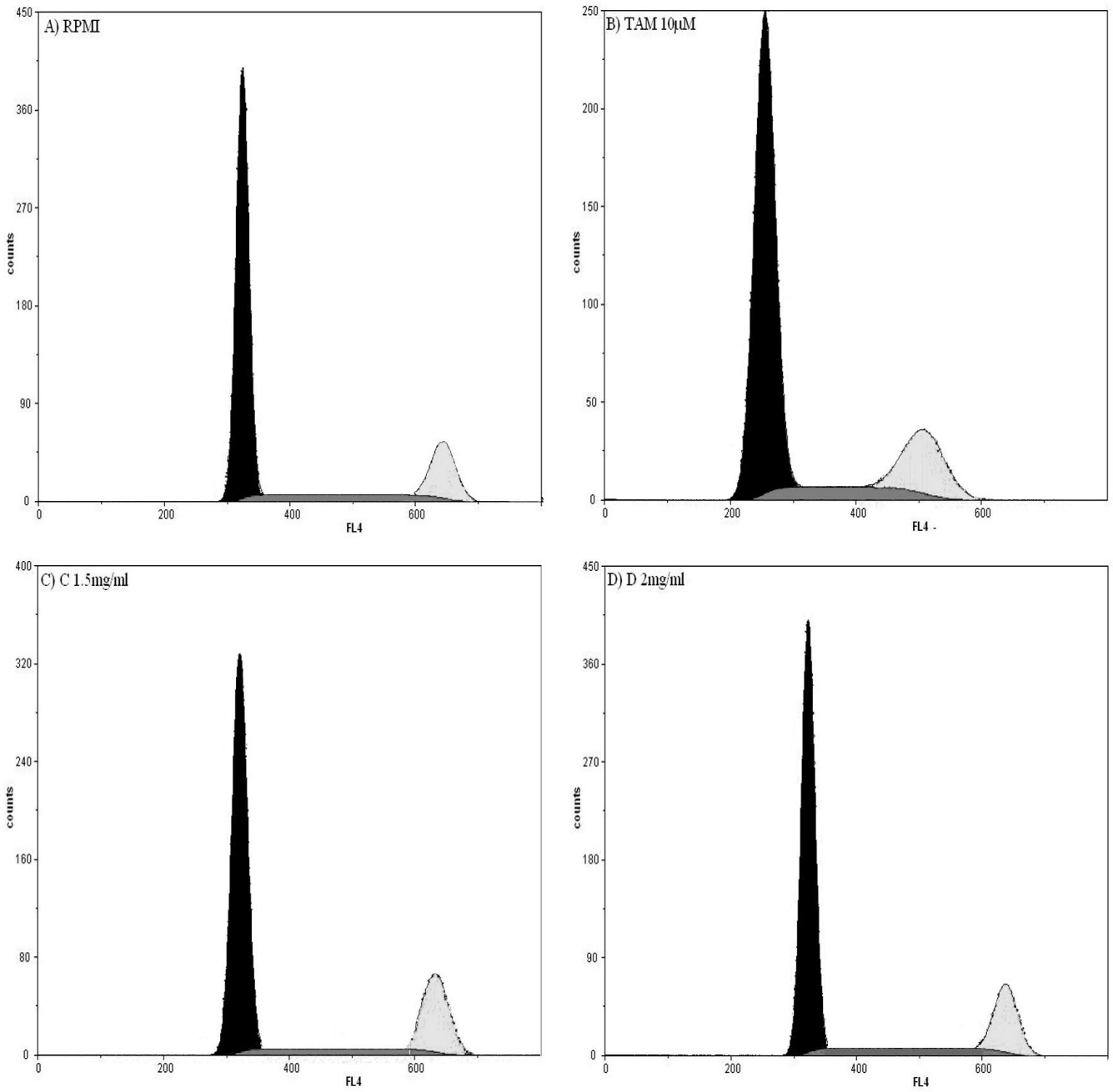

Figure 4. Cell cycle pattern and flowcytograms of cells treated with RPMI, Tamoxifen 10 µM, aerial (C, 1.5 mg/ml) and root (D, 2 mg/ml) extracts and stained with DAPI reagent show G1, S, G2/M phases of cell cycle under different assay conditions. A (RPMI), B (TAM 10 µM), C (C, 1.5 mg/ml), D (D, 2 mg/ml).

carcinogenesis (Singh et al., 2002). There are a number of herbs that have shown the ability to induce cell cycle arrest and to play an important role in cancer prevention and therapy. Genistein, daidzein and isoflavonoids in soybean are thought to play an important role in breast cancer prevention (Wang et al., 2002; Chen et al., 2003). Two other examples of natural compounds with anti-cancer properties are quercetin and apigenin. Quercetin is one of the major flavonoids found in the human diet exerts a dose dependent inhibitory effect on cell proliferation with cell cycle arrest in G2/M phase. Quercetin has also been shown to inhibit cell proliferation in colon carcinoma (HCT-116 and HT-29) and mammary adenocarcinoma (MCF-7) cell lines after 24 h of exposure (Ramos 2007; Ong et al., 2004). Apigenin a flavone found in celery has antiproliferative effect with cell cycle arrest in G2/M in MCF-7 cells and induction of caspase activities in HL-60 cells (Yin et al., 2001; Wang et al., 1999).

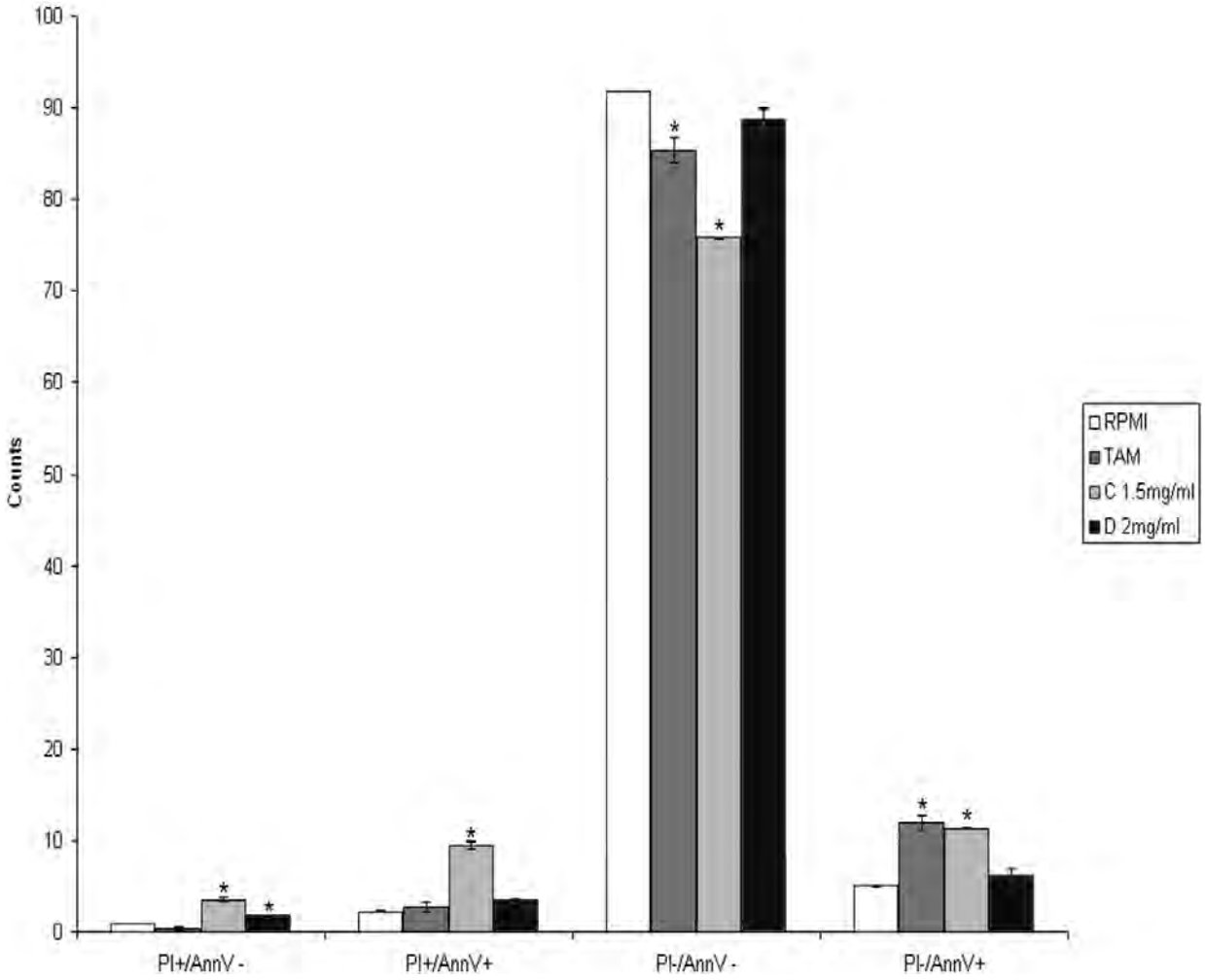

Figure 5. Apoptosis induction of ***A. orientalis*** extracts on T47D cells. Cells were plated in six well plates 48 hours and treated with RPMI and Tamoxifen 10µM controls and plant extracts at IC_{50} concentrations. After 48 hours of treatment, both adherent and floating cells were harvested and washed with PBS and then incubated with Annexin V-FITC and PI. Data on 10,000 cells were acquired and processed using a PARTEC flowcytometer with FloMax software. The data represent the mean±SE of 3 independent experiments. *Mean differences with $p< 0.05$ were considered statistically significant

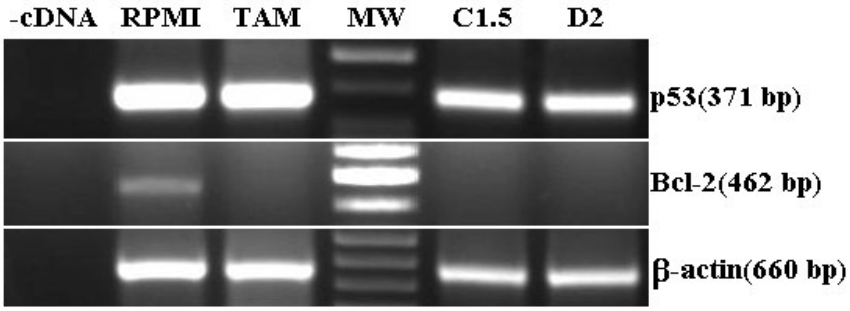

Figure 6. Effects of IC_{50} concentrations of ***A. orientalis*** aerial and root methanolic extracts in comparison to RPMI and Tamoxifen 10 µM on the mRNA levels of p53 and Bcl-2 in T47D cells. After 48 h incubation with IC_{50} concentrations of extracts, total RNA was isolated from treated and control samples and expression alterations of genes were analyzed using RT-PCR. cDNA (RT-PCR without complementary DNA) TAM (Tamoxifen 10 µM), MW (Marker), C1.5 (aerial methanolic extract 1.5 mg/ml), D2 (root methanolic extract 2 mg/ml).

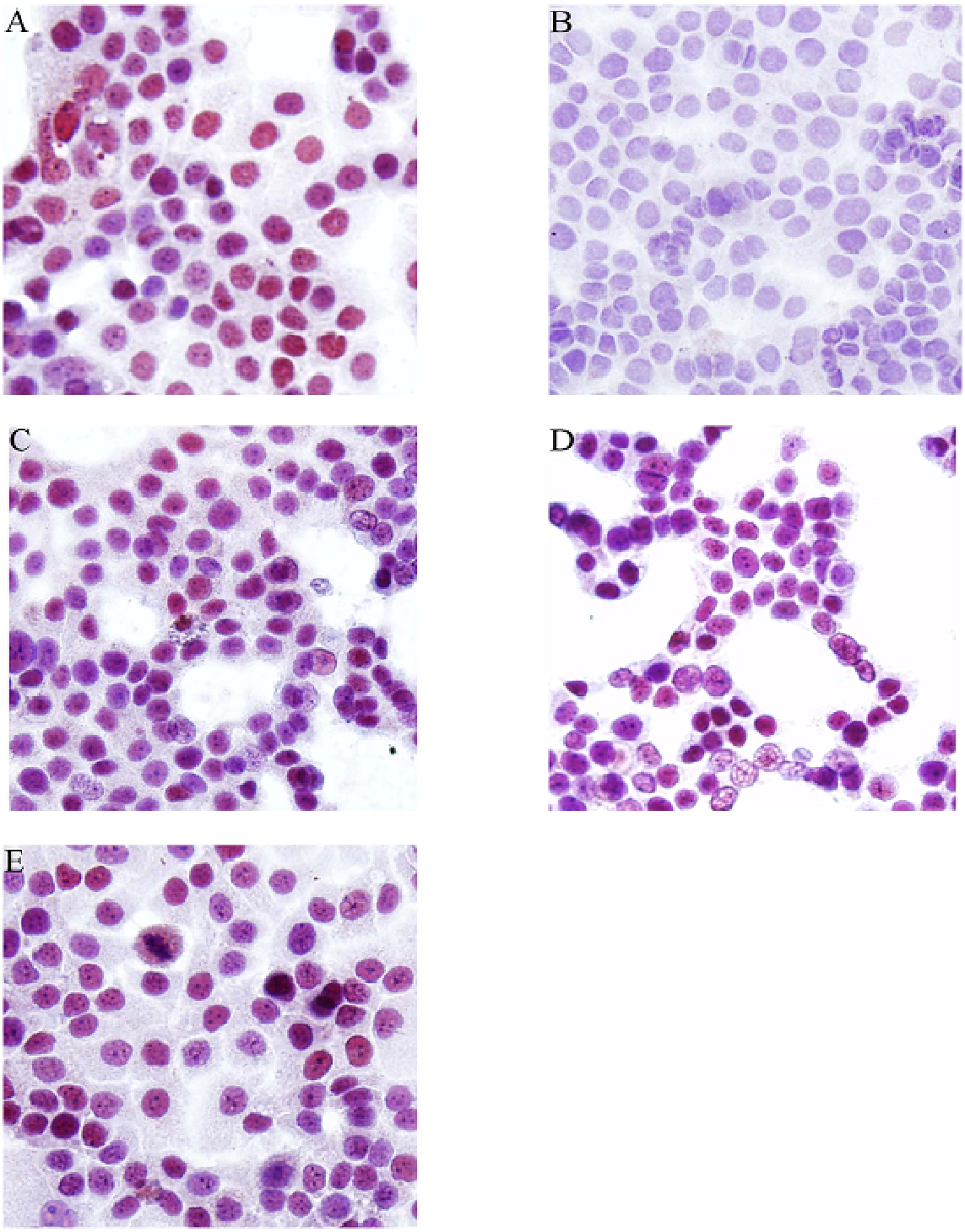

Figure 7. Immunostaining of p53 protein in T47D cells. Cells were immunostained with primary antibody for p53 and AEC chromogen, mounted and studied under light microscope (magnification 400X). A (RPMI), B (negative control), C (C, 1.5 mg/ml), D (D, 2 mg/ml), E (TAM 10 µM)

Ethnopharmacological data has been one of the common useful ways for the discovery of biological active compounds from plants. Methanolic extracts were normally used for anticancer screening because traditional practitioners believed that mostly the polar compounds were responsible for the claimed anticancer properties. At the mechanistic level, one of the most attractive and promising approaches in this quest for new and improved

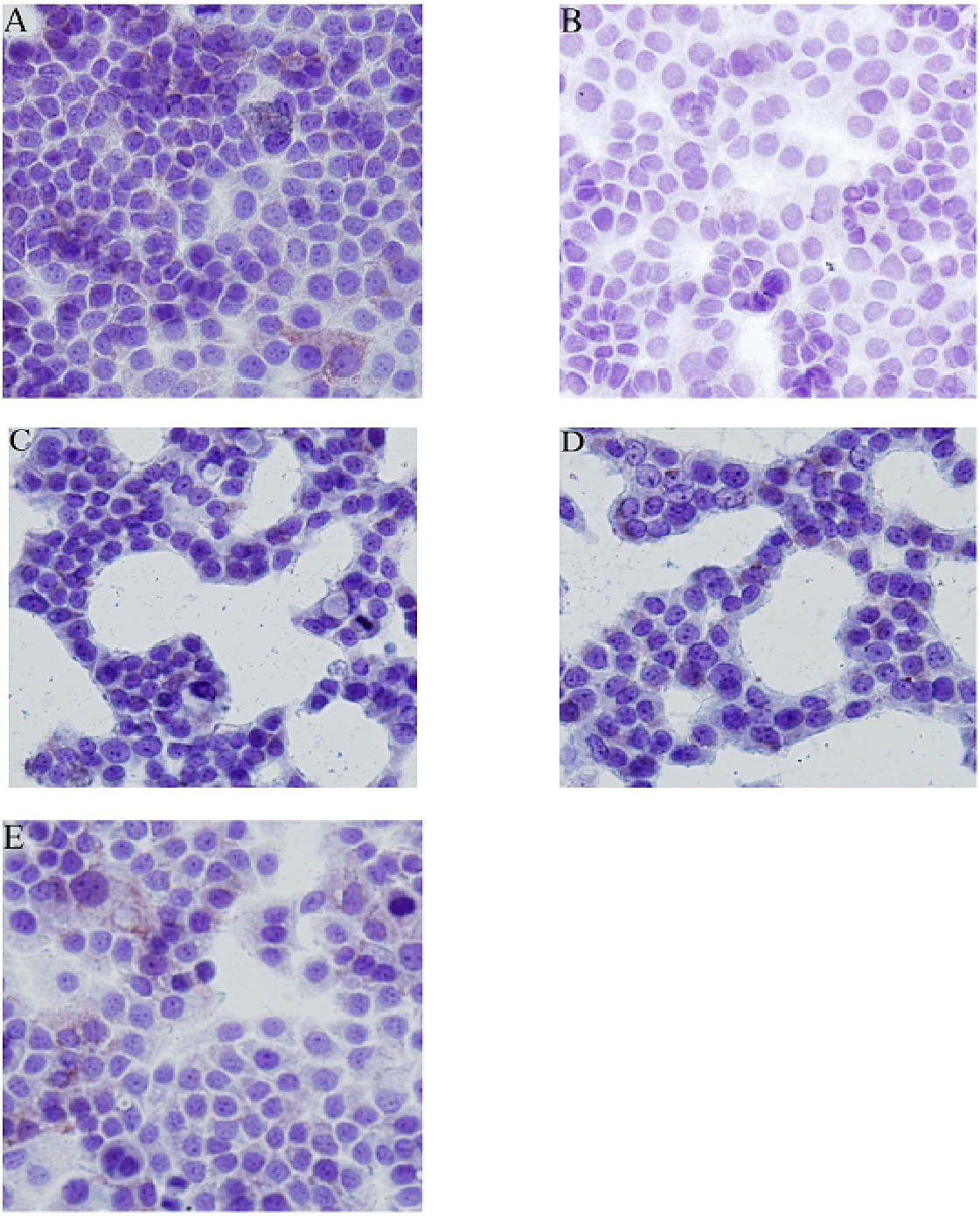

Figure 8. Immunostaining of Bcl-2 protein in T47D cells. Cells were immunostained with primary antibody for Bcl-2 and AEC chromogen, mounted and studied under light microscope (magnification 400X). A (RPMI), B (negative control), C (C, 1.5 mg/ml), D (D, 2 mg/ml), E (TAM 10 µM).

cytotoxic anticancer agents consists in the gene and protein expression alteration of some important genes such as p53 and Bcl-2. Abnormalities of the p53 tumor suppressor gene are among the most frequent molecular events in human and animal neoplasia. It is now accepted that the inactivation of this gene, as a result of mutation, is a key step in neoplastic transformation and progression. This effect is usually related to the loss of p53 activated cell death, growth arrest, and/or control of genomic stability. However, it is not yet completely clear which of the many properties of p53 are particularly important in ontogenesis. In addition, the p53 activation

system may be influenced by cell type and specific tissue effects. Mutations of the p53 gene are observed in about 30-50% of breast cancers. The expression of this oncoprotein in breast cancers seems to be related to poor prognosis associated with a high histological grade, epidermal growth factor receptor positivity, and Bcl-2 and oestrogen receptor negativity (Megha et al., 2002; Tokino and Nakamura, 2000). The Bcl-2 oncoprotein is a 26 kDa integral membrane protein localized to the membranes of the endoplasmic reticulum, mitochondria, and nuclear envelope. It can function to suppress or delay the induction of apoptosis in a number of systems, including prostate, skin, lymphoid tissues, and mammary gland (Yang et al., 2003).

In conclusion, extracts of ***A. orientalis*** have concentration and time dependent antiproliferative activities with alteration on cell cycle pattern in comparison to control RPMI. Extract of aerial in comparison to the extract of root part shows higher antiproliferative activity. Also both extracts induced apoptotic cell death in T47D cells that the effect of aerial part is considerable. In the presence of plant extracts the p53 and Bcl-2 gene expression and also nuclear staining of p53 and cytoplasmic staining of Bcl-2 were decreased. Results suggest that the methanol extracts of ***A. orientalis*** may contain bioactive compound that kill T47D breast carcinoma cell by apoptosis mechanism. The activities of this plant may be due to the presence of coumarin compounds that occurred in family of ***Umbelliferae***. The finding of new chemical structures from ***Astrodaucus*** plants provides a fascinating novel resource for anti-cancer drugs. Further molecular studies are undergoing to elucidate the mechanism(s) of action of these extracts on cancer cells. Thus we are surveying these effects on various fractions especially on coumarin fraction, and also isolating the purified compounds for making this study complete for the present time.

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Abbreviations: AEC, 3-Amino-9-ethylcarbazole; **ATCC**, American type culture collection; **DAPI,** 4, 6-diamidine-2-phenylindole dihydrochloride; **DEPC,** diethylpyrocarbonate; **DMSO,** dimethyl sulfoxide; **dNTP,** PCR nucleotide mix; **DTT,** dithiothreitol; **FBS,** fetal bovine serum; **FITC,** fluorescein isothiocyanate; **IC_{50},** the median inhibitory concentration; **M-MuLV**, reverse transcriptase; **MTT,** 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide; **PBS,** phosphate buffer solution; **Pen-strep,** penicillin-streptomycin; **PI,** propidium iodide; **TAM,** tamoxifen; **triPure,** RNA, DNA and protein isolation reagent.

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