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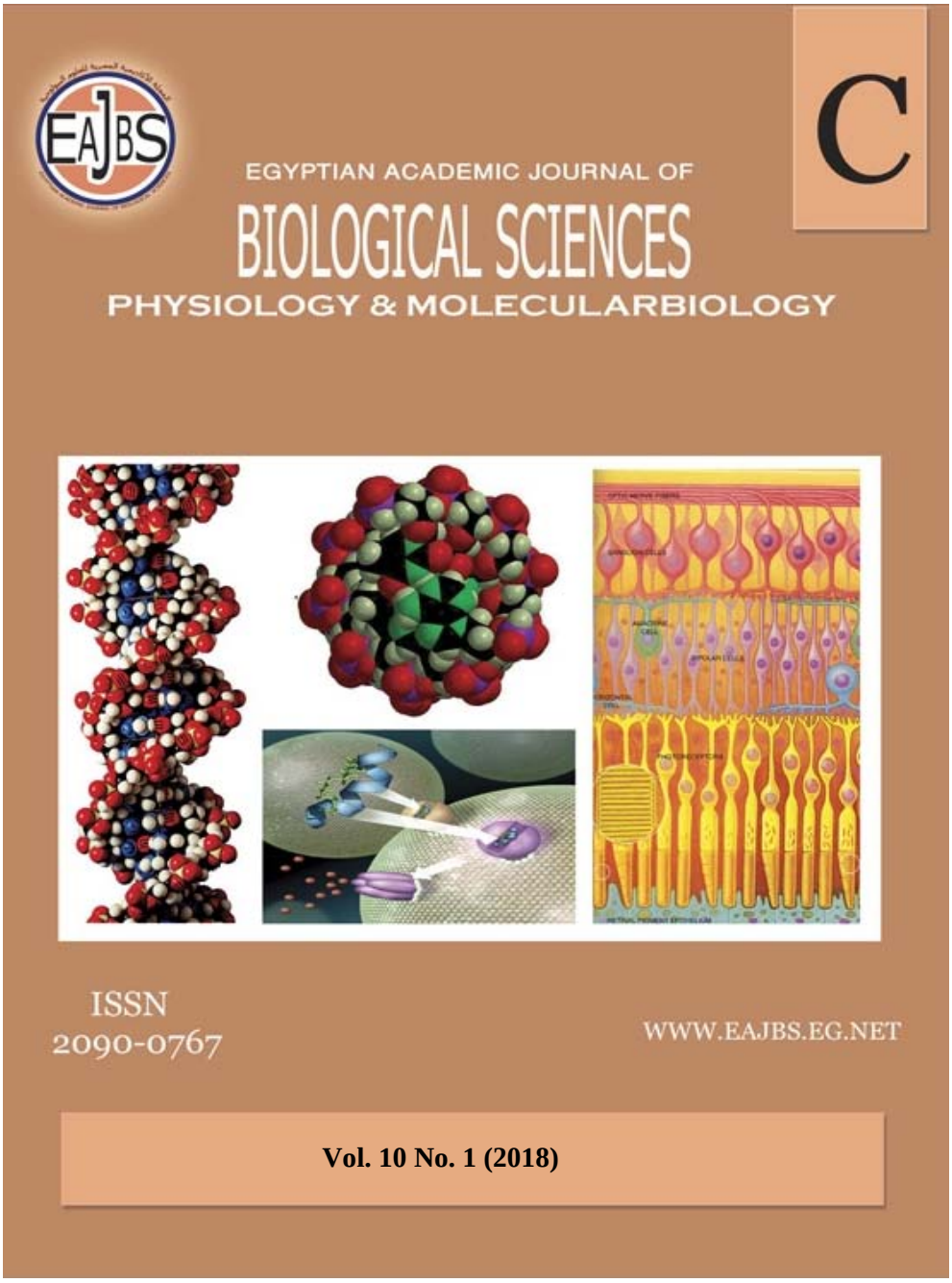

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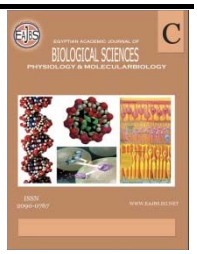

In vitro Antiproliferation Effect of *Atriplex halimus* L. Crude Extract on Human Cell Lines by Induction of Apoptosis and G2/M phase Arrest.

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ABSTRACT

Current clinical ways include the usage of medicinal plants as therapeutic agents in a large scale of applications. The present study is focused on the anticancer activity of the methanolic extract of *Atriplex halimus* and the likely underlying mechanisms were also investigated. Results exhibited that the extract had an antiproliferative effect and highly cytotoxicity in cancer cells, Human hepatocellular carcinoma (HepG2) (IC50 = 54.86 µg/ml), against the human breast adenocarcinoma cell line (MCF-7) with IC50 value of 153.6 µg/ml and lung cancer cell line (A549) (IC50 = 101.9 µg/ml). In contrast, this extract exhibited no induces growth inhibition (did not cytotoxic activity) on normal cell lines. Flow cytometric analysis of propidium iodide staining detected that the treatment of HepG2 cells with *A. halimus* led to increase G2/M phase cell cycle arrest. The data obtained from acridine orange/ethidium bromide (AO/EtBr) protocol showed the morphological characters of apoptosis such as apoptotic bodies, nuclear fragmentation and chromatin condensation; in addition, necrotic cells were observed. The real time-PCR and western blotting techniques were used to measure the mRNA levels of p53, Bax, and Bcl-2 genes and proteins expression. The apoptotic process triggered by *A. halimus* involved the upregulation of p53 and Bax and the downregulation of Bcl-2 in both techniques. These data indicated that *A. halimus* exhibited antiproliferative effect by a cell cycle blocking at the G2/M phase and apoptosis mediated cytotoxicity in carcinoma cells. In conclusion, these results suggest that *A. halimus* could be a good candidate species as a natural source of anticancer agents.

INTRODUCTION

First of all, the use of medicinal plants has known to humans for along times, but that use has been over increased in the last three decades. Nearly, more than 80% of people over the world depend on them for some part of primary healthcare (Ekor, 2013).

Medicinal plants are rich with natural products which have biological activities, cost-effectiveness, easy accessibility and safety (Ahmad *et al*., 2017).*Atriplex halimus* L. belongs to family Amaranthaceae (formerly Chenopodiaceae), is a shrub species, woody stems and triangular leaves. It is growing naturally in Mediterranean region, Sinai, Arabia and East Africa (Boulos, 1999). In addition, *A. halimus* is wide ecological amplitude due to its distribution in arid, semi-aride and salty regions (xero-halophyte species) (Khaldi *et al*., 2015). Also, it can be cultivated under alkaline and saline conditions as complementary forage (Chikhi *et al*., 2014). *Atriplex halimus* have many medicinal uses; to treat chest ailments, as a laxative, for intestinal worms, to cure stomach pains, anti-inflammatory, regulate gallbladder excretion (Houérou *et al*., 1992; Tahar *et al*., 2017) treating muscular pain and intestinal diseases in animals (Chicki *et al*., 2014).

Moreover, the *A. halimus* extracts have antioxidant activity. This power is probably due to the high salinity that contains the plant (Khaldi *et al*., 2015). The crude extract and fractions of *A. halimus* growing in Egypt have cytotoxic activity against MCF-7 and PC3 carcinoma cells (Amal, 2016).

Also, the cytotoxicity effects of *Atriplex confertifolia* on *HeLa* cells (human cervical cancer cells) were investigated (Welch, 2004). More than 94% of the *HeLa* cells were killed using the most bioactive fraction of *A. confertifolia*. Capua *et al*., (2010) reported that extracts of *A. confertifolia* induce apoptotic cell death.

One of the helpful strategies for anticancer drug development is the induction of apoptosis (programed cell death) in cancer cells (Hu and Kavanagh 2003). The apoptosis plays a significant role in deleting the mutated hyper proliferating cells from the system. Thus, inducers of apoptosis have been utilized in cancer medication and stimulation of apoptosis paths is a key mechanism by which cytotoxic medicines kill tumor cells and now being considered as an important technique for measurement of the clinical efficiency of some anti-cancer drugs (Earnshaw *et al*., 1998).

Cho *et al*., (2005) reported that the control of cell division is the major regulatory mechanism of cell growth; the cell cycle analysis is a novel and suitable method for cancer control and eradication. There is the relationship between cell cycle and apoptosis, and together play an important role in the sensitivity of cancer cells to chemotherapy, whereas, accumulation of cells in subG1 phase was an indication of apoptosis. (Haruyo *et al* 2006).

Medicinal plants induce process of apoptosis which is involved by different tumor suppressor genes including p53. The p53 gene induces apoptotic cell death by direct or indirect change expression of Bcl-2 family of proteins, Bcl-2 and Bax (Reed, 1999 & Levine *et al*., 1991). The Bcl-2 gene is an anti-apoptotic gene that suppresses initiation steps of apoptosis via inhibition of the pro-apoptotic proteins (Youle and Strasser 2008). The p53 gene may modulate susceptibility of cells to apoptosis by down regulation of *BCL-2* and be causing up-regulation of *BAX* (Choi *et al*., 2000).

The present study aims at evaluating the effect of crude extract of the halophyte *Atriplex halimus* L. (Amaranthaceae), on *In vitro* human cell growth. The possible underlying antiproliferation mechanisms were investigated, by studying the effect of *A. halimus* on cell cycle arrest, cell apoptosis and apoptosis-related genes and proteins of the HepG2 cell line.

MATERIAL AND METHODS

Plant Collection and Extract Preparatio:

Atriplex halimus plant was collected from Wadi Gharandal,South Sina, Egypt. Herbs were washed and shade dried for a week and was milled to the fine powder. For the antiproliferation effect analysis, extracts were obtained by soxhlet extraction at a ratio of 20 g dry powder in 200mL of ethanol. They were kept for 48 h at 4∘C, filtered through a Whatman n∘4 filter paper, evaporated under vacuum. After drying under vacuum, the powder was dissolved in DMSO to get 2.5% (2.5mg powder in 100 μL DMSO) as stock concentration.

Assessment of Cytotoxicity and Cell Proliferation:

Cell proliferation and viability of the cells were estimated through the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) colorimetric assay (Mossman 1983 & Edmondson *et al*., 1988). This method is depend on the metabolic decrease of soluble MTT by enzyme activity of mitochondrial of viable tumor and normal cells, into an insoluble color formazan product, which can be measured spectro photometrically after resolving in dimethylsulfoxide (DMSO). The crude extract was further diluted in medium to produce five concentrations (10, 25, 50, 100, 200 µg/ml). The three selected cancer cell lines and two normally used in this proposal was derived from human breast adenocarcinoma cells (MCF-7), Human hepatocellular carcinoma (HepG2), lung cancer cell line (A549), normal liver (THLE2) cells and normal lung fibroblast cell line (Wi38). *A. halimus* was added with different concentrations for 24 hours at 37 °C in a 5% CO_2 with 95% humidity incubator. In addition, various cisplatin concentrations as reference chemotherapeutic drug were added and the microplates were incubated for a further 48 hour in DMEM medium (200 µL). Wash the medium gently two times with ice-cold PBS and add a volume of 200 µL MTT (Molecular probes, Eugene, Oregon, USA; Cat.no.V-13154)] to each well. The microplate was incubated at 37 °C for another 4 hours in CO_2 incubator. About 180 µL medium/MTT was removed and 100 µL of acidified isopropanol were added per well to solubilize the formazan produced. Finally, incubate the microplate with shaking for 15 minutes. The absorbance of each well was measured at 630 nm using a microplate reader (ELX800, Biokit, spain). Sigmoidal and dose-dependent curves were designed to plot the experiment results of the. The concentration of the compounds inhibiting 50 % of cells (IC_{50}) was calculated using this sigmoidal curve.

Cell Cycle Analysis by Propidium Iodide (PI) Staining Using Flow Cytometry:

To assess the crude extract of *A. halimus* on the cancerous cell division (HepG2), the cells were digested with warm Trypsin-EDTA + warm PBS-EDTA (0.25%) (500 µl + 500 µl) with incubation for 10 minutes at 37°C. The mixture was centrifuged 450 rpm for 5 min, and then the supernatant was carefully removed. The mixture was washed twice in warm PBS and the cell pellet was re-suspend in 500 µl warm PBS, centrifuged and supernatant was removed. A volume of 150 µl PBS + 350 µl Ice-cold absolute ethanol (final cons of EtOH is 70%) was added, mixed with a pipette then vortexed several times, and incubated at 4°C for 1 hour to fix the cells. To remove ethanol, the mixture was centrifuged at 350 rpm for 10 minutes and then the supernatant was carefully removed. The mixture was washed twice in warm PBS and the cell pellet was re-suspend in 500 µl warm PBS, centrifuged and the supernatant was removed. The pellet was re-suspended in

100 µl PBS and was stored at 4° for up to 4 days. In the darkness, the cells were stained with 100 µl of PI solution + 50 µl RNase A solution (100 µg/ml), and incubated in darkness for 30-60 min. The stained cells were read in Attune flow cytometer (Applied Bio-system, US).

Statistical analysis. The significance of any differences between groups was evaluated using Student's *t-test*. P values of less than 0.05 were considered significant. All values in tables were expressed as mean ± SD.

Analyses of apoptotic cells by *EtBr/AO* double staining:

The differant morphology of the cells was showed after staining with ethidium bromide/acridine orange (EtBr/AO) by fluorescence microscopy. The HepG2 cells (4×104 cells/ml) cultured in the presence or absence of the extract were collected and washed in cold PBS. Then the EtBr/AO solution (1:1 v/v) was added to the cell suspension at a final concentration of 100 µg/ml and then they were incubated at room temperature for 5 min. The stained cells were estimated under a fluorescent microscope.

Determination of the Expression Levels of Apoptosis-Regulatory Genes:

Total RNA was isolated from HepG2 cells using Gene JET RNA Purification Kit (Thermo Scientific, # K0731, USA) according to the manufacturer's protocol. Total RNA (5µg) was reverse transcribed using Revert Aid H Minus Reverse Transcriptase (Thermo Scientific, #EP0451, USA) to produce cDNA. The cDNA was used as a template to determine the relative expression of the apoptosis-related genes using StepOnePlus real time PCR system (Applied Biosystem, USA).

The primers were designed by Primer 5.0 software and their sequences were as follow: p53 was 5'-CCCAGGTCCAGATGAAG-3', and the reverse was 5'-CAGACGGAAACCGTAGC-3'. The forward primer for Bcl-2 was 5'-GGATGCCTTTGTGGAACTGT-3', and the reverse was 5'-AGCCTGCAGCTTTGTTTCAT-3'. And Bax was 5'-TTTGCTTCAGGGTTTCATCC-3', and the reverse was 5'-CAGTTGAAGTTGCCGTCAG A-3'. The housekeeping gene β-actin was used as a reference to calculate fold change in target gene expression. A 25-µL PCR mix was prepared by adding 12.5 µL of 2X Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, # K0221, USA), 2 µL of cDNA template, 1 µL forward primer, 1 µL reverse primer, and 8. 5 µL of nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 min, 40-45 cycles of amplification of DNA denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s. At the end of the last cycle, the temperature was increased from 63 to 95 °C for melting curve analysis. The cycle threshold (Ct) values were calculated for target genes and the housekeeping gene, and relative gene expression was determined using 2-ΔΔCt method .

Statistical Analysis:

All results were expressed as means ± standard error (SE). The statistical significance was evaluated by one-way ANOVA using SPSS 18.0 software. Values were considered statistically significant when $P \leq 0.05$. Comparison of means was carried out with Tukey's Honestly Significant Difference (Tukey's HSD) test.

Western Blotting:

The HepG2 cells were lysed in RIPA buffer and the protein concentration was determined by the Bradford method. Equal volumes of protein were loaded and separated on a 10% SDS-PAGE gels. Proteins were transported to a 0.45 µm polyvinylidene fluoride membrane (Millipore). After incubation with the primary antibodies overnight at 4°C, membranes were incubated with horseradish peroxidase

(HRP)-conjugated goat anti-rabbit antibodies (1: 5,000; Santa Cruz Biotechnology, Inc.) for 1 hr at room temperature. The specific protein bands were developed using tetramethylbenzidine (TMB, Sigma Chemical Company, St. Louis, MO, USA). The density of each band was normalized by β-actin. Sources and dilution factors of primary antibodies were TGFb (1:150; Bioworld), phosphatidylinositol-3-kinase (PI3K, 1:100, abcam), and ERK (1:100, Santa Cruz Biotechnology).

RESULTS

Anti-Proliferous Activity of *A. halimus* Crude Extract Using MTT Assay:

The anticancer activity of *A. halimus* crude extract was screened on multiple cell lines and examined by MTT assay that exhibited cytotoxic activity against human breast adenocarcinoma cell line (MCF-7) with the IC_{50} value of 153.6 µg/ml, as shown in Figure 1. Furthermore, figure 2 showed that *A. halimus* crude extract inhibited the proliferation of human hepatocellular carcinoma (HepG2) (IC50 = 54.86 µg/ml), but no cytotoxic activity was noticed against normal liver (THLE2) cells (IC50 = 6797 µg/ml) (Fig. 2). Moreover, this extract also exhibited promising cytotoxic activity against lung cancer cell line (A549) (IC50 = 101.9 µg/ml). Like in THLE2, the crude extract of *A. halimus* did not induce growth inhibitory effect on normal lung fibroblast cell line (Wi38) (IC50 = 3457 µg/ml) was examined (Fig. 3).

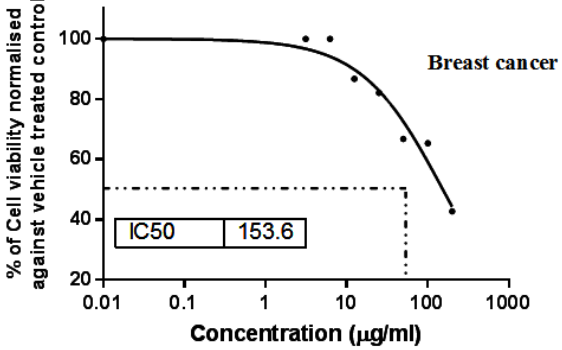


Fig. 1. Anti-proliferation effect of *A. halimus* crude extract on MCF-7 (breast cancer) cell development. MTT method is used for measuring the viability of MCF-7 cells. The number of surviving cells is expressed as the mean ± SD.

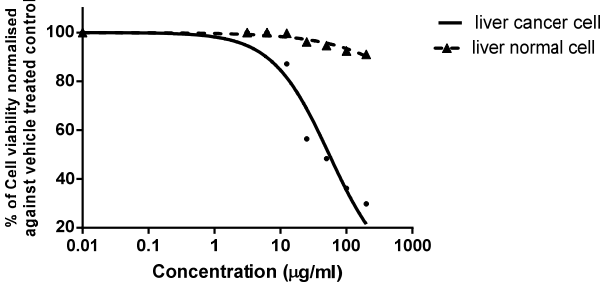


Fig. 2. Anti-proliferation effect of *A. halimus* crude extract on HepG2 (liver cancer) and THLE2 (liver normal) cell development. MTT assay is used for measuring the viability of the HepG2 and THLE2 cells. The number of surviving cells is expressed as the mean ± SD.

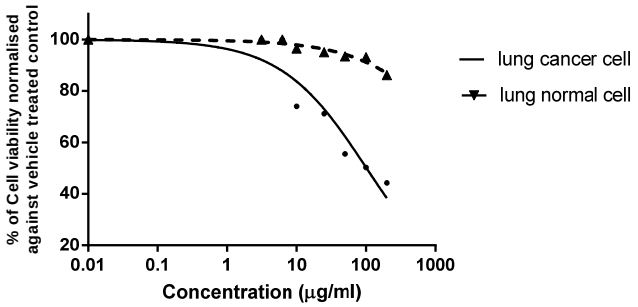


Fig. 3. Anti-proliferation effect of *A. halimus* **crude extract** on A549 (lung cancer cell line) and Wi38 (lung normal) cell development. MTT test is used for measuring the viability of the A549 and Wi38 cells. The number of surviving cells is expressed as the mean ± SD.

Effect of *A. halimus* Crude Extract on Cell Cycle Arrest Utilizing Flow Cytometry:

According to the inhibition rate of liver cancer (HepG2) cell viability, we want to confirm that anti-proliferative effect of *A. halimus* was associated with cell cycle arrest using flow cytometry-based cell cycle distribution. We found that compared with the control group, crude extract at 54.86 μg/ml affected the cell cycle distribution on HepG2 cells (Fig. 4). The G0/G1 phase showed decreased from 60% to 33%, while the S-phase percentage increased slightly from 19% to 21% in the control and crude extract of *A. halimus,* respectively. Interestingly, the percentage of HepG2 cells at the G2/M phase was increased after incubation with *A. halimus* extract (46%) as compared to the control (21%). These results suggested that *A. halimus* inhibited the cellular proliferation of HepG2 cells via G2/M phase arrest of the cell cycle.

Induction of Apoptotic Cells by Acridine Orange-ethidium Bromide *(*AO/EtBr) Immunofluorescence Staining Double Staining:

To verify the apoptotic nature of the cell death caused by crude extract of *A. halimus* we evaluated the nuclear changes by EtBr/AO double staining technique. Apoptotic activity from *A. halimus* ethanolic crude extract was evaluated with respect to the morphology of cells by fluorescence microscopy. Compared with spontaneous apoptosis which was observed in control cells, HepG2 treated with 54.86 μg/ml from the extract showed an increase of apoptosis. Regarding to the negative control, the nuclear region of viable cells is uniformly green. Whereas, early apoptotic cells are orange with bright dots corresponding to nuclear chromatin fragmentation. Late apoptosis cells showed condensed red nucleus. Cells treated with *A. halimus* extract exhibited characteristic changes of apoptosis e.g. fragmentation and formation of apoptotic bodies, as showed in figure (5).

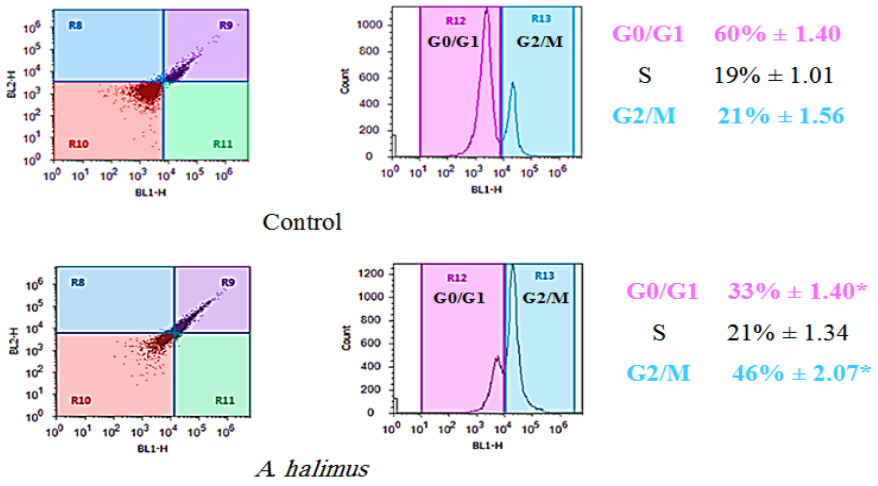
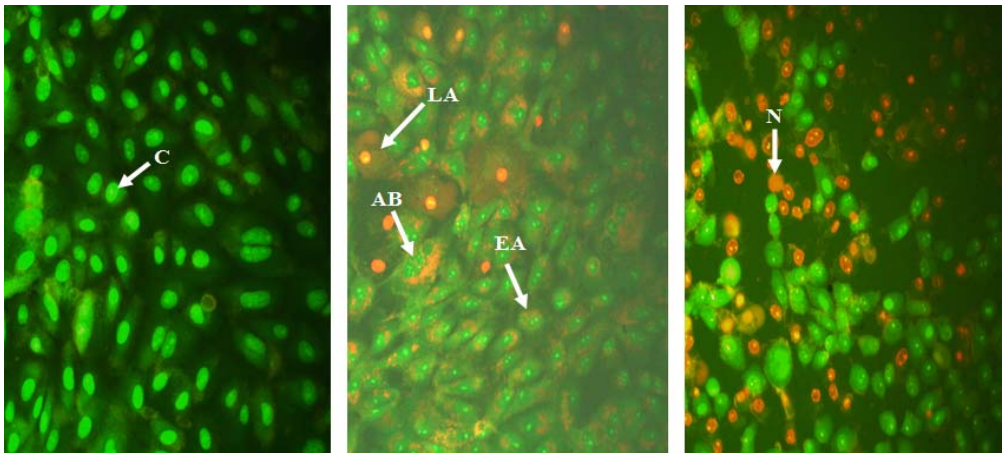


Fig. 4. Effect of crude extract of *A. halimus* on the cell cycle arrest. Liver cancer cells (HepG2) were treated with extract at the concentration of 54.86 µg/mL in order to check the cell cycle distribution and then were analyzed by *flow cytometry.*
*P < 0.05 against control.



HepG2 cells untreated **HepG2 cells treated with *A. halimus* crude extract**

Fig. 5. Effects of *A. halimus* ethanolic extract on apoptosis induction among HepG2 cells, extract treated by 54.86 µg/mL and stained with AO/EB. The images were taken using fluorescence microscopy at 20×. C: control cells showed green normal nucleus, no treated cells; EA: early apoptosis cells showed green bright nucleus with fragmented chromatin; LA: late apoptosis cells showed condensed red nucleus; AB: apoptotic body; N: necrosis.

Expression Level of Apoptosis-related Genes:

To evaluate the molecular mechanism of *A. halimus*-induced apoptosis in HepG2 cells, the expression level of apoptosis-related genes such as *p53*, *Bcl*-2 and *Bax* in HepG2 cells which were estimated by real-time PCR (qRT-PCR). Results detected that compared to the untreated group, figure (6) illustrated that the expression level of p53 and Bax were increased. In contrast, the expression level of *Bcl*-2 gene was decreased. Thus, the results indicate that the extract killed HepG2 cells through apoptosis mechanism mainly via the expression of p53, Bcl-2, and Bax genes.

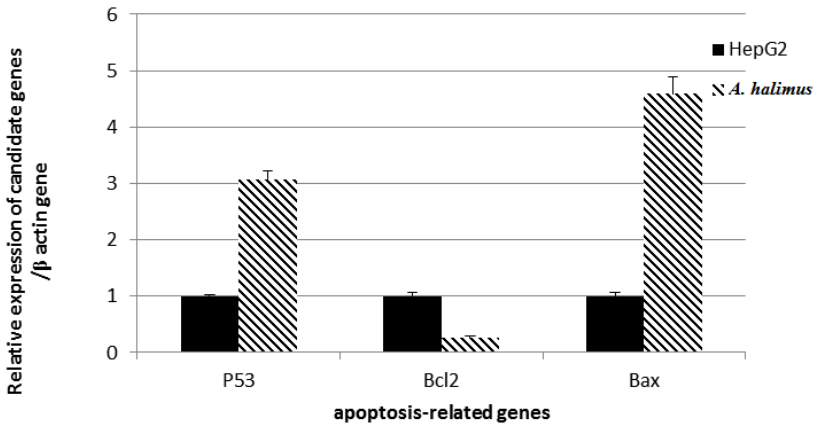


Fig. 6. Effects of *A. halimus* ethanolic extract on apoptosis-related genes After exposure to 54.86 μg/mL, mRNA expression of p53, Bcl-2, and Bax was assessed by quantitative RT-PCR $^{*}P < 0.05$, compared to the control group.

Effects of *A. halimus* Ethanolic Extract on the Expression of Apoptosis-related Proteins in HepG2 Cells:

To determine the mechanism of *A. halimus*-induced apoptosis related proteins of HepG2 cells, the protein expression levels of p53, Bcl-2, and Bax were verified by western blotting (Fig. 7). The p53 and Bax, proteins were clearly up-regulated as compared to the control, whereas the Bcl2 protein expression level was down-regulated decreased by the extract.

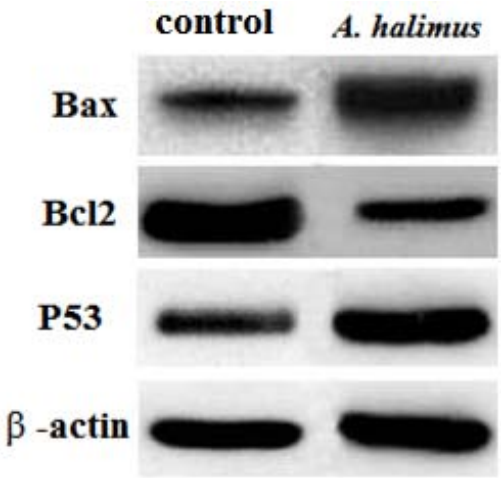


Fig. 7. An effect of *A. halimus* ethanolic extract on protein expression of p53, Bcl-2, and Bax was evaluated by western blotting in HepG2 cells compared with the control group.

DISCUSSION

These results revealed that the *A. halimus* ethanolic crude extract may not affect normal cells, in contrast, induced cytotoxicity on cancer cell lines according to a more intense decrease in cell viability on human cancer cell lines. Thus, this result suggested that *A. halimus* has antiproliferative effect with various concentrations of ethanolic crude on different cancer human cell lines.

These results agree with Boulaaba *et al*., (2013), who exhibited the antiproliferative effect of the methanolic extract from *Arthrocnemum indicum* on human colon cancer (Caco-2) cells. The *A. indicum* inhibited the Caco-2 cell growth in a dose-dependent manner. Moreover, Amal (2016), evaluated the cytotoxic effect of *A. halimus* on different human cell lines such as, Caco (intestinal carcinoma), HELA (cervical carcinoma), HEP2 (larynx carcinoma), HCT (colon carcinoma), A549 (lung carcinoma), HepG2 (hepatocellular carcinoma), MCF-7 (breast carcinoma) and PC3 (prostate carcinoma), and found that reduction in cells viability of all the human cancer cell lines were used, thus, she reported that *A. halimus* has cytotoxic activities.

This results harmony with Seham *et al*., (2014) who found that *Atriplex lindleyi* Moq. growing in Egypt had highly cytotoxicity against the four studied carcinoma cell lines (breast, colon, hepatocellular and lung) using the *in vitro* (MTT).

Our findings showed that HePG2 cells were blocked at the G2/M phase following 24 h exposure to *A. halimus* crude extract at 54.86 μg/mL. This was already observed in colon cancer (Caco-2) cells treated with *Arthrocnemum indicum* extract, and, suggested that *A. indicum* may be useful as a candidate source of antiproliferation molecules (Boulaaba *et al*., 2013). Similar results were observed by Wu *et al*., (2006) who found the growth inhibited, arrested the cell cycle at the G2/M phase and induced apoptosis using a novel and synthetic anticancer agent, the enediyne derivative THDA these results suggest that THDA may be useful as one of the investigational compounds in the treatment of leukemia.

Our results are in agreement with those of Farha *et al*., (2013), who reported that analysis of cell cycle the induction of apoptosis by accumulating cells in G1 phase and G2/M cell cycle arrest. Anti-cancer compounds arrest the cell cycle at the G1, S, or G2/M phase and then induce apoptotic cell death pathway. In addition, the function of apoptotic cell death is either removing irreparable or unrepaired damaged cells (Chen *et al*., 2017).

The method Acridine orange/ Ethidium bromide (AO-EB) staining has been mentioned in studies of apoptosis in human lymphoid. This application based on differential uptake and DNA staining of AO and EB, equilibrium low concentrations (Liegler *et al*., 1995). The AO/EB procedure uses to detection of apoptosis and can differentiate among early apoptotic, late apoptotic and lifeless cells. OA/EB is a more convenient and economical method (Liu *et al*., 2015).

Acridine orange is a dye that stains both live and lifeless cells; Ethidium bromide only stains dead cells that have miss membrane integrity. Live cells stain uniformly green. In contrast, early apoptotic cells stain green and contain bright green dots in the nuclei. Late apoptotic cells also incorporate EtBr and show condensed and display condensed and fragmented orange chromatin. On the other hand, EtBr penetrated into the membranes of necrotic cells and stains their nuclei orange color, but present nuclear morphology resembling that of viable

cells (Farha *et al*., 2013; Sahar *et al*., 2016).

This result agrees with Monga *et al*., (2013), who used AO/EB immunofluorescence staining to apoptosis cells detection and showed that *Acacia catechu* ethanol extract has anti-proliferation effect on HepG2 cells and SCC-25 cells via apoptosis induction (Lakshmi *et al*., 2017).

In this study, the mRNA expression levels of apoptotic-related genes and proteins, p53, Bax and Bcl-2 in HepG2 cells treated with the *A. halimus* extract were investigated. Our results showed that the *A. halimus* induces apoptosis which is elicited through p53, Bax and Bcl-2 genes. This finding is in agreement with many studies those demonstrated the role of p53, Bax and Bcl-2 in inducing apoptosis (Youle and Strasser 2008).

CONCLUSION

Based on the findings of the study, the crude extract of *A. halimus* had killing activity was specific for human tumor cells, without much toxicity on the normal cell. These results indicated that *A. halimus* has antiproliferative effect. Moreover, *A. halimus* crude extract evaluated to identify the mechanisms behind the toxicity. Cell cycle blocking at the G2/M phase with apoptotic cells was provided by AO/EB dual staining. Gene expression of RNA and protein studies confirm *A. halimus* extract to induction of apoptosis via activation of p53, and Bax and inactivated of Bcl2.Taken together, these data suggest that *A. halimus* could be a candidate species as a source of anticancer molecules.

REFERENCES

Ahmad A., Tandon S., Xuan T. D. and Nooreen Z. (2017). A Review on Phytoconstituents and Biological activities of Cuscuta species. Biomed Pharmacother. 92, 772-795.

Amal M. K. (2016). Macroscopic and microscopic charachterization of atriplex halimus l. Growing in Egypt and in vitro evaluation of its cytotoxic activity. world journal of pharmacy and pharmaceutical sciences. 5, 6, 84-100.

Boulaaba M., Khaoula M., Soninkhishig T., Junkyu H., Abderrazak S., Kiyokazu K., Riadh K., Hiroko I., and Chedly A. (2013). *In Vitro* Antiproliferative Effect of *Arthrocnemum indicum* extracts on Caco-2 Cancer Cells through Cell Cycle Control and Related Phenol LC-TOF-MS Identification. Evid Based Complement Alternat Med. 2013:529375. doi: 10.1155/2013/529375.

Bouls L. (1999). Flora of Egypt, Vol. I. Al Hadara publishing Cairo, Egypt. Pp 419.

Capua C. J., Nick P. H., C. Malcolm M. S., Johnston G. R., O'Neill L. K., Schaalje G. B., Christopher M. L., and Gary M. B.. (2010). Cytotoxicity of *Atriplex confertifolia*. Hindawi Publishing Corporation Journal of Toxicology. Volume 2010, Article ID 976548, 7 pages.

Chen Y., Yi Q., Li L., Jing C., Xu Z., and Yubo X. (2017). Morphine Can Inhibit the Growth of Breast Cancer MCF-7 Cells by Arresting the Cell Cycle and Inducing Apoptosis. Biol. Pharm. Bull. 40, 1686–1692.

Chikhi I., Allali H., El Amine Dib M., Medjdoub H. and Tabti B. (2014). Antidiabetic activity of aqueous leaf extract of *Atriplex halimus* L. (Chenopodiaceae) in streptozotocin–induced diabetic rats. Asian Pacific J Trop Dis.; 4(3): 181–4.

Cho S. H., Toouli C. D., Fujii G. H., Crain C., and Parry D. (2005). Chk1 is essential for tumor cell viability following activation of the

replication checkpoint. Cell Cycle, vol. 4,1, 131–139.

Choi J. H., Ahn K. S., Kim J. and Hong Y. S. (2000). Enhanced induction of Bax gene expression in H460 and H1299 cells with the combined treatment of cisplatin and adenovirus mediated *wt*-p53 gene transfer. Exp Mol Medicine 32: 23–28.

Earnshaw W. C., Martins L. M. and Kaufmann S. H. (1998). Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem. 68:383–424.

Edmondson J., Armstrong L. and Martinez A. (1988). A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. J Tissue Cult. Methods 11:15-17.

Ekor M. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol; 4: 177.

Farha A. K., Geetha B. S., Mangalam S. N., Dhanya S. R., Latha P. G. and Remani P. (2013). Apoptosis mediated cytotoxicity induced by isodeoxyelephantopin on nasopharyngeal carcinoma cells. Asian Journal of Pharmaceutical and Clinical Research. Vol 6, Suppl 2, 51-56.

Haruyo I., Mangalam S. N.,Yasunari T., Alan Sheeja D. B., Suresh K., Oommen V. O., and Bharat B. A. (2006). Isodeoxyelephantopin, a novel sesquiterpene lactone, potentiates apoptosis, inhibits invasion, abolishes osteoclastogenesis through suppression of Nuclear Factor k B activation and Nuclear Factor k B regulated gene expression. Clinical Cancer Research; 12(19): 5910-5918.

Houérou H. N. (1992). The role of saltbushes (*Atriplex spp*.) in arid land rehabilitation in the Mediterranean Basin: a review. Agrofor Syst, ;18 (2): 107–48.

Hu W. and Kavanagh J. (2003). Anticancer therapy targeting the apoptotic pathway. Lancet Oncol; 4: 721-729.

Khaldi A., Amamra D., Tir touil A., Maghdouri N. and Belhadj N. (2015). Effects of *Atriplex Halimus* Resistant Bacterial strain of Different origins. International Conference on Advances in Agricultural, Biological & Environmental Sciences (AABES-2015) July 22-23, London (UK).

Lakshmi T., Devaraj E., Upendra N. and Rajagopal V. (2017). *Acacia catechu* Ethanolic Seed Extract Triggers Apoptosis of SCC-25 Cells. Pharmacognosy Magazine; 13(Suppl 3): S405–S411.

Levine A. J., Momand J. and Finlay C. A. (1991). The p53 tumour suppressor gene. Nature; 351: 453-456.

Liegler T. J., william H., Benedict T. S. and daniel P. S. (1995). Detection and Quantification of Live, Apoptotic, and Necrotic Human Peripheral Lymphocytes by Single-Laser Flow Cytometry. Clinical and Diagnostic Laboratory Immunology. p. 369–376.

Liu K., Peng-cheng L., Run L. and Xing W. (2015). Dual AO/EB Staining to Detect Apoptosis in Osteosarcoma Cells Compared with Flow Cytometry.

Monga J., Saurabh P., Rajinder S. C., Chetan S. C., Shailender S. C. and Manu S. (2013). Growth Inhibition and Apoptosis Induction by (+)-Cyanidan-3-ol in Hepatocellular Carcinoma. PLoS ONE 8(7): e68710. doi:10.1371/ journal. pone. 0068710.

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

Reed J. C. (1999). Dysregulation of apoptosis in cancer. J Clin Oncol; 17:2941-2953.

Sahar B., Karim E., Mahmoud M., and Ali H. (2016). *Primula auriculata* Extracts Exert Cytotoxic and Apoptotic Effects against T-29 Human Colon Adenocarcinoma Cells. Iranian Journal of Pharmaceutical Research, 15 (1): 311-322.

Seham M. A., Bassem M. M., Gamila M. W., Khaled A. and Marwa, M. M. (2014). Screening of some Plants in Egypt for their Cytotoxicity against four Human Cancer cell lines. International Journal of Pharm Tech Research.Vol.6, No.3, pp 1074-1084.

Tahar S. B., Mahfoud H-M., Mshvildadze P., V., Mohamed Y. (2017). Study of the Enzymatic and Anti-Inflammatory Activities of Phenolic Extracts of *Atriplex halimus L.* and *Haloxylon scoparium* Pomel. Der Pharma Chemica, 9 (1):40-45.

Welch N. R., (2004). Cytotoxicity of *Atriplex confertifolia*: isolation and dose response of anticancer compounds in a plant indigenous to Western North America, M.S. thesis, BYUHonors, Provo, Utah, USA,.

Wu Z.-Z., Ching-Ming C., Sheng-Huei Y., Yi-Hsiung L., Xiu-Wei H., Yu-Jhang L., Ming-Jung W. and Shinne-Ren L. (2006). Induction of G2/M phase arrest and apoptosis by a novel enediyne derivative, THDA in chronic myeloid leukemia (K562) cells. Molecular and Cellular Biochemistry 292: 99–105.

Youle R. J and Strasser A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol; 9:47-59.