ANTI-CANCER EFFECTS OF *Zygophyllum album* AND *Suaeda palaestina* EXTRACTS ON HUMAN LIVER CANCER CELL LINES

MARWA, M. EL-ATTAR[1], F. M. ABDEL-TAWAB[2], A. A. AWAD[2], EKRAM S. AHMAD[3], H. A. KAMEL[1] AND AMAL I. HASSAN[1]

1. *Radioisotopes Department, Atomic Energy Authority, Dokki, Giza, Egypt*
2. *Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*
3. *Department of Cell Biology, National Research Centre, Dokki, Giza, Egypt*

Traditional medicines derived mainly from wild plants, still play major roles in the management of various diseases (Karunanayake and Tennekoon, 1993; Narayana and Dobriyal, 2000). Medicinal plants are currently used as raw materials for extraction of active ingredients which are used in the synthesis of several drugs including treatment of cancer disease (Rasool, 2012). In addition, the World Health Organization (WHO) has reported that about 80% of the world population relies on conventional medicine for their primary health care (Latif *et al*., 2014). Out of a total of 250,000 plant species present on earth, approximately one thousand showed anticancer activities (Bibi *et al*., 2012).

Cancer is one of the most dangerous diseases in humans and presently there is a considerable amount of new anticancer agents derived from natural products like medicinal plants (Sharma, *et al*., 2011). Globally, about 1 in 6 deaths is due to cancer, whereas cancer is the second leading cause of death worldwide, as well as it is responsible for an evaluated 9.6 million deaths in 2018 (WHO, 2018). The potential of using medicinal plants as anticancer drugs was recognized in 1950's by U.S National Cancer Institute (NCI). Since 1950, major contributions have been made for finding out naturally existing anticancer drugs (Sharma *et al*., 2011). Moreover, about 60% of the actually used anticancer drugs have been extracted from natural products, mostly of plant origin (Latif *et al*., 2014)

Zygophyllum album is a halophyte shrubby plant belonging to Zygophyllaceae family Täckholm and Boulos, (1977) which includes about 27 genera and 285 species frequently restricted to arid and semiarid areas (Beier *et al*., 2003). Indeed, many plants belonging to this genus have anti-inflammatory, molluscicidal, and expectorant activities (Hassanean *et al.*, 1993). Furthermore, Ksouri *et al*. (2013) reported that dichloromethane fraction of the halophytic species; *Zygophyllum album* had cytotoxic activity on cells viability particularly lung (A549) and colon (DLD-1) cancer cell lines. These finding resorted that these halophytic species have appreciable antitumour activity in the less polar extracts. Belmimoun *et al*. (2017) reported that *Z. album* had antioxidant, anti-inflammatory,

Egypt. J. Genet. Cytol., 48:77-90, January, 2019
Web Site (*www.esg.net.eg*)

and antimicrobial activities. Besides, *Z. album* decreased significantly the rate of DNA fragmentation and the incidence of micronucleated polychromatic erythrocytes compared with doxorubicin treated group in mice (Ezz El-Din *et al*., 2018).

Suaeda palaestina (*S. palaestina*) is a halophyte shrubby plant belonging to the Chenopodiaceae family, which is an annual herbaceous plant particularly abundant in the Mediterranean salt marshes. Hypoglycaemic and hypolipidaemic activities have been reported in this family. Patra *et al*. (2011) stated that *S. maritima* is a rich source of natural antioxidant with moderate antimicrobial activities. On the other hand, the dichloromethane extract of *Suaeda fruticosa* showed the highest anticancer activity against human lung carcinoma (A549) and colon adenocarcinoma cell lines (DLD-1, Caco-2 and HT-29) with specificity against DLD-1 (IC50 = 10±1 µg/ml). These findings demonstrated the remarkable potentiality of this edible halophyte as valuable source of antioxidants which exhibit original and interesting anti-inflammatory and anticancer capacities (Oueslati *et al*., 2012).

The cyclin B1–CDK1 complex is a key regulator of mitotic entry (Nigg, 2001). A large number of proteins are phosphorylated by the cyclin B1–CDK1 complex prior to mitotic entry, which initiates the mitotic events, including nuclear envelope breakdown, centrosome separation, spindle assembly, and chromosome condensation (Heald and McKeon, 1990; Peter *et al*., 1990; Nigg *et al*., 1996). Inactivation of CDK1 kinase activity by degradation of cyclin B1 is required for both mitotic exit and DNA replication in the following cell cycle progression. Multiple layers of regulation of the mitotic events are linked to the control of the activity of the cyclin B1-CDK1 complex in a number of situations to make cells enter mitosis, arrest at G2-phase, or skip mitosis (Nakayama and Yamaguchi, 2013).

Apoptosis is a crucial pathway for regulating homeostasis, responding to DNA damage and controlling cell proliferation. Apoptotic cells including cancerous cells undergo a natural process of dying (Mahani *et al*., 2015). The induction of apoptosis in cancer cells is one of the most helpful mechanisms for the development of drugs used in the treatment of cancer (Hu and Kavanagh, 2003). Apoptosis plays a crucial role in getting rid of the hyperprolife rating mutated cells from the system.

Medicinal plants induce the process of apoptosis which is involved in different tumor suppressor genes including *p53* (Brady and Attardi, 2010). It also plays a critical role in the regulation of cell cycle arrest in the G2/M phase and is involved in DNA repair during activation of ribonucleotide reductase (Levine, 1997). Apoptosis could be stimulated by *p53* gene through down regulation of *Bcl-2* gene, and activation of caspases (Haupt and Haupt, 2004). It has been known that *p53* contributed to the transcriptional acti-

vation of large numbers of target genes (Mirzayans *et al*., 2012).

The present study aims to assess the effects of dichloromethane crude extracts of the halophyte *Z. album* (*Zygophyllaceae*) and *S. palaestina* (*Chenopodiaceae*), on *in vitro* human cancer cell lines growth. The possible underlying antiproliferation mechanisms were investigated, by studying the effects of *Z. album* and *S. palaestina* on cell cycle arrest, regulatory and apoptosis-related genes of the HepG2 cell line.

MATERIALS AND METHODS

1. *Plant collection and extract preparation*

Fresh whole plants were collected from South Sinai, and Borg El-Arab, Alexandria (Egypt), respectively, in March 2015 by Desert Research Center (DRC) Egypt. These halophytes were identified at the Flora and Phyto-Taxonomy Research Section, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt. The plant materials were dried at ambient temperature and stored in a dry place prior to use. The plant samples were washed well with water, dried at room temperature in the dark, and then ground in an electric grinder to give a coarse powder. Shoot powders (50 g) were extracted by dichloromethane solvent. The powders were soaked in 150 ml solvent at room temperature for 48h. The plant extracts were collected drop wise and filtered using Whatman No. 1 filter paper. The residues were soaked in 150 ml of solvent for 24 h and were filtered again. The extracts were then dried and finally placed in glass vials and stored at -20°C and the extracts were re-suspended in 1% dimethyl sulphoxide (DMSO) before testing (Moustafa *et al*., 2014).

2. *Cell lines*

Human hepatocellular carcinoma cancer cell line (HepG2) obtained from the American Type Culture Collection (ATCC), Manassas, Virginia, USA, that was used in the present study. The cells, treated with half-maximal inhibitory concentration (IC50), were 27.74 µg/ml for *Z. album*, and 30.76 µg/ml for *S. palaestina* according to a pervious study (El-Attar *et al*., 2019).

3. *Cell cycle analysis by propidium iodide (PI) staining using flow cytometry*

To assess effect crude extract of *Z. album* and *S. palaestina* on the cancerous cell division in HepG2, the cells were treated with plant extracts and were digested with warm Trypsin-EDTA + warm Phosphate-buffered saline (PBS)-EDTA (0.25%) (500 µl + 500 µl) with incubation for 10 minutes at 37°C. The mixture was centrifuged at 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-suspended in 500 µl warm PBS, centrifuged and the 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°C for up to 4 days. In 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).

4. Determination of the expression levels of regulatory and apoptosis-inducing 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 (5 µg) 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 (Table 1). 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 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec). 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 $2^{-\Delta\Delta CT}$ method.

RESULTS AND DISCUTION

1. Effect of Z. album and S. palaestina crude extract on cell cycle arrest utilizing Flow Cytometry

In a previous study (El-Attar *et al*., 2019) the results at the *in vitro* level, indicated that *Zygophyllum album* and *Suaeda palaestina* extracts induced anti-proliferative effects and high cytotoxicity on human cancer cell lines and no cytotoxic activity on normal cell lines. The half-maximal inhibitory concentration (IC50) observed in the human hepatocellular carcinoma (HepG2) was 27.74 µg/ml in *Z. album*, while IC50 in *S. palaestina*, it was 30.76 µg/ml. After realizing the high inhibition rate of HepG2 on cell viability by *Z. album* and *S. palaestina*, we tempted to assess possible cytotoxicity effects of both the tested extracts on cell cycle arrest using flow cytometry based on cell cycle

checkpoints. Comparison of 27.74 µg/ml of *Z. album* treated cells to the control group has shown that the cell cycle distribution of HepG2 cells was indeed affected (Fig. 1). The G0/G1 phase showed a reduction from 66% to 34%, and also the S-phase from 20% to 19% in the control and *Z. album* treated cells, respectively. On the other hand, the percentage of HepG2 cells at G2/M phase highly increased for *Z. album* treatment to be about 3.35 folds when compared to the control, as G2/M phase increased from 14% to 47%. The cell cycle distribution of HepG2 cells treated with 30.76 µg/ml of *S. palaestina*, was also affected. The G0/G1 phase decreased from 66% in control to 31% treated cells and the S-phase was reduced from 20% to 18% (Fig. 2). The percentage of G2/M phase in *S. palaestina* treatment to be about 3.6 folds when compared to the control in the same phase, as G2/M increased from 14% to 51%. These results showed significant accumulation of treated HepG2 cells in the G2/M phase, and confirmed that *Z. album* and *S. palaestina* have profound cytotoxic effects via induction of G2/M phase arrest of the cell cycle.

Many investigators have shown that different cytotoxic agents such as radiation and some medicinal plants can induce G2/M phase accumulation; G2/M arrest is followed by cell death. Multiple cytotoxic molecules induce mitotic cell death (apoptosis) which occurs in parallel with G2/M arrest (Bonelli *et al*., 1996; Sleiman and Stewart, 2000; Wu *et al*., 2006; Gismondi *et al*., 2013). Al-Senosy *et al*. (2018) found that *Atriplex halimus* extract resulted in G2/M arrest and apoptosis in treated human hepatocellular carcinoma cell line (HepG2). *Moringa oleifera*, a medicinal plant, was found to induce G2/M arrest by increasing the expressions of genes related to G2/M such as *p53*, and *p21*. Furthermore, it stimulated apoptosis via decreased anti-apoptotic *Bcl-2* expression, while it increased the expressions of *caspases 3* and *7* (Gismondi *et al.*, 2013; Adebayo *et al.*, 2017).

2. Determination of the expression levels of cell cycle regulatory gene and apoptosis-regulatory genes.

2.1. Effect of Z. album and S. palaestina treatments on relative expression of Cyclin B1 and CDK1 regulatory genes in HepG2 cells

The expression levels of *cyclin B1* and *CDK1* genes in HepG2 were determined by quantitative real time-PCR. Figs (3 and 4) and Tables (2 and 3) showed that, compared to the control (untreated) HepG2 cells, the expression levels of *cyclin B1* and *CDK1* were down-regulated at a concentration equivalent to their IC50, *Z. album* (27.74 µg/ml) and *S. palaestina* (30.76 µg/ml) for 24 h. The results indicated that both *Z. album* and *S. palaestina* triggered G2/M arrest involved transcriptional suppression.

The cell cycle is mediated by the activation of a highly conserved protein kinase family, the cyclin-dependent kinases (CDKs) (Stewart *et al.*, 2003). Cyclins can activate CDKs by forming complexes

with Cdks, and these cyclin/CDK complexes are cell cycle regulators. Among them, the cyclin B1/CDK1 complex, in which B-type cyclins associate with CDK1, is considered as one of the primary regulators of transition from the G2 to M phase. This complex was originally discovered and defined as the maturation-promoting factor or M phase-promoting factor (MPF) (Shangguan *et al*., 2014). The cell cycle arrests at the G2 phase and mitosis cannot occur without activation of the cyclin B1/CDK1 complex. In the present study, flow cytometric analysis showed that *Z. album* and *S. palaestina* caused G2/M phase cell cycle arrest, suggesting the occurrence of sequential events of cell cycle arrest followed by apoptosis.

2.2. Determination of the expression levels of apoptosis-regulatory genes

In order to determine the expression level of pro-apoptotic and anti-apoptotic genes in treated cells, the mRNA levels of *P53*, *caspase-3* and *Bcl-2* were evaluated by qRT-PCR. After 24 h treatment with IC50 concentrations of the *Z. album* and *S. palaestina*, *P53* expression was upregulated in HepG2 cells, with 1.78-fold and 2.87-fold, respectively, (compared to untreaed control). In addition, *caspase-3* expression was up-regulated in HepG2 cells, with 2.08-fold after *Z. album* treatment and 5.46-fold after *S. palaestina* treatment. On the other hand, the expression level of *Bcl-2* mRNA was markedly down-regulated in HepG2 cell lines after treatment by *Z. album* (0.24-fold) and *S. palaestina* (0.55-fold) (Tables 4 and 5 and Figs 5 and 6).

These results revealed that *Z. album* and *S. palaestina* enhanced the activity of pro-apoptotic *P53*, tumour suppressor gene; which is critically involved in cell cycle regulation, DNA repair, and programmed cell death. In addition, they stimulated the activity of *caspase-3*, which evidently serves as a marker of programmed cell death. On the other hand, anti-apoptotic *Bcl-2*, which contributes to cancer formation and progression by promoting the survival of altered cells, decreased at a concentration equivalent to their IC50. All the above indicating that both plants extract triggered the progression of apoptosis to circumvent the possible occurrence of carcinogenic effects.

Evaluation of *p53*, *caspase-3*,and *Bcl-2* expression is a common approach used for the analysis of apoptosis upon treatment with related compounds. This was in partial agreement with the results reported by Al-Senosy *et al*. (2018) and Mitupatum *et al*. (2016).

Finally, based on the aforementioned results, the crude extracts of *Zygophyllum album* or *Suaeda palaestina* proved to have anti-proliferative effects and induced cell cycle arrest at the G2/M phase and apoptosis in human cancer cell lines. These data suggest that both extracts could be considered as new effective and promising herbal therapeutic agent for human cancer treatment.

SUMMARY

The present study aims to the assessment of anticancer activity of the extracts of the two medicinal plants *Zygophyllum album* and *Suaeda palaestina*., in a previous study, human liver cancer cell lines (HepG2) showed the cytotoxicity of the two plants by MTT assay. The half-maximal inhibitory concentration (IC50) observed in the (HepG2) was 27.74 µg/ml in *Z. album*, while IC50 of *S. palaestina* was 30.76 µg/ml. According to IC50 of both plants, the flow cytometric analysis was investigated and indicated that the treatment of HepG2 cells with *Z. album* and *S. palaestina* led to substantial elevation of G2/M phase cell cycle arrest. The real time-PCR was used to measure the mRNA levels of *cyclin B1* and cyclin dependent kinase (*CDK1*) genes. Both genes showed down-regulation of expression levels in HepG2 with *Z. album* and *S. palaestina* compared to the untreated cells. The apoptotic mechanism activated by both plant extracts resulted in up-regulations of *p53* and *Caspase 3* and the down-regulation of *Bcl-2* on mRNA levels on HepG2 after treatment with both extracts. In conclusion, these results suggest that *Z. album* and *S. palaestina* could be considered as candidate species being a good natural source of anticancer agents.

REFERENCES

Adebayo, I. A., W. G. Balogun and H. Arsad (2017). *Moringa oleifera*: An apoptosis inducer in cancer cells. Tropical Journal of Pharmaceutical Research, 16: 2289-2296.

Al-Senosy, Neima K., A. Abou-Eisha and E. S. Ahmad (2018). Cytotoxic effects of *Atriplex halimus* extract on human cancer cell lines. Bioscience Research, 15: 1718-1728.

Beier, B. A., M. W. Chase and M. Thulin (2003). Phylogenetic relationships and taxonomy of subfamily *Zygophylloideae* (*Zygophyllaceae*) based on molecular and morphological data. Plant Systematics and Evolution, 240: 11-39.

Belmimoun, A., A. M. Touil, B. Meddah and P. Sonnet (2017). Antibacterial translocation effect of methanolic extract of *Zygophyllum album* from Algeria on infected model rats with *Bacillus cereus*. Int. J. Biosci., 10: 108-119.

Bibi, F., M. Yasir, G. C. Song, S. Y. Lee and Y. R. Chung (2012). Diversity and characterization of endophytic bacteria associated with tidal flat plants and their antagonistic effects on oomycetous plant pathogens. The Plant Pathology Journal, 28: 20-31.

Bonelli, G., M. C. Sacchi, G. Barbiero, F. Duranti, G. Goglio, D. C. Verdun, J. S. Amenta, M. Piacentini, C. Tacchetti and F. M. Baccino (1996). Apoptosis of L929 cells by etoposide: a quantitative and kinet-

ic approach. Exp. Cell Res., 228: 292-305.

Brady, C. A. and L. D. Attardi (2010). *p53* at a glance. J. Cell Sci; 123: 2527-2532.

El-Attar, Marwa M., A. A. Awad, F. M. Abdel-Tawab, H. A. Kamel, Ekram S. Ahmad and Amal I. Hassan (2019). Assessment of cytotoxic and anticancer activity of *Zygophyllum album* and *Suaeda palaestina* extracts on human liver cancer cell lines. In press.

Ezz El-Din, A. E., Maaly, H. M. Ali; Ekram, S. Ahmed, M. M. M. Hassanane and W. K. B. Khalil (2018). Ameliorative effect of *Zygophyllum album* extract against hepatotoxicity induced by doxorubicin in male mice. International Journal of Pharmacology, 14: 421-427.

Gismondi, A., L. Canuti, S. Impei, G. Di Marco, M. Kenzo, V. Colizzi and A. Canini (2013). Antioxidant extracts of African medicinal plants induce cell cycle arrest and differentiation in B16F10 melanoma cells. International Journal of Oncology, 43: 956-964.

Hassanean, H. A., M. M. A. El-Hamouly, S. A. El-Moghazy and D. W. Bishay (1993). 14-Decarboxyquinovic and quinovic acid glycosides from *Zygophyllum album*. Phytochemistry, 33: 667-670.

Haupt, S. and Y. Haupt (2004). Manipulation of the tumor suppressor *p53* for potentiating cancer therapy. In Seminars in Cancer Biology, Academic Press, 4: 244-252.

Heald, R. and F. McKeon (1990). Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. Cell, 61: 579-589.

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

Karunanayake, E. H. and K. H. Tennekoon (1993). Search of novel hypoglycemic agents from medicinal plants. Diabetes Mellitus and its Complications, An Update: 192-205.

Ksouri, W. M., F. Medini, K. Mkadmini, J. Legault, C. Magné, C. Abdelly and R. Ksouri (2013). LC-ESI-TOF-MS identification of bioactive secondary metabolites involved in the antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Zygophyllum album* Desf. Food Chemistry, 139: 1073-1080.

Latif, A., H. M. Amer, M. E. Hamad, S. A. R. Alarifi and F. N. Almajhdi (2014). Medicinal plants from Sau-

di Arabia and Indonesia: *In vitro* cytotoxicity evaluation on Vero and Hep-2 cells. Journal of Medicinal Plants Research, 8: 1065-1073.

Levine, A. J. (1997). *p53*, the cellular gatekeeper for growth and division. Cell, 88: 323-331.

Mahani, E. S., B. Ebrahimi, M. Abbasnejad, B. Rasoulian, and V. Sheibani (2015). *Satureja khuzestanica* prevents the development of morphine analgesic tolerance through suppression of spinal glial cell activation in rats. Journal of Natural Medicines, 69: 165-170.

Mirzayans, R., B. Andrais, A. Scott and D. Murray (2012). New insights into p53 signaling and cancer cell response to DNA damage: implications for cancer therapy. BioMed Research International, 1: 1-16.

Mitupatum, T., K. Aree, S. Kittisenachai, S. Roytrakul, S. Puthong, S. Kangsadalampai, and P. Rojpibulstit (2016). mRNA expression of *Bax*, *Bcl-2*, *p53*, *Cathepsin B*, *Caspase-3* and *Caspase-9* in the HepG2 cell line following induction by a novel monoclonal Ab Hep88 mAb: cross-talk for paraptosis and apoptosis. Asian Pac. J., Cancer Prev., 17: 703-712.

Moustafa, Seham M., B. M. Menshawi, G. M. Wassel, K. Mahmoud and Marwa M. Mounier (2014). Screening of some plants in Egypt for their cytotoxicity against four human cancer cell lines. Int. J. Pharm. Tech. Res., 6: 1074-1084.

Nakayama, Y. and N. Yamaguchi (2013). Role of cyclin B1 levels in DNA damage and DNA damage-induced senescence. In International Review of Cell and Molecular Biology, Academic Press, 305: 303-337.

Narayana, D. A. and R. M. Dobriyal (2000). In Pharmacology and therapeutics in the new millennium. Complimentary Medicines and 21st Century Therapeutics: Challenges for Pharmacologists. Narosa Publishing House New Delhi., 28 p. 332.

Nigg, E. A. (2001). Cell division: mitotic kinases as regulators of cell division and its checkpoints. Nature Reviews Molecular Cell Biology, 2: 21-32.

Nigg, E. A., A. Blangy and H. A. Lane (1996). Dynamic changes in nuclear architecture during mitosis: on the role of protein phosphorylation in spindle assembly and chromosome segregation. Experimental Cell Research, 229: 174-180.

Oueslati, S., R. Ksouri, H. Falleh, A. Pichette, C. Abdelly and J. Legault (2012). Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halo-

phyte *Suaeda fruticosa* Forssk. Food Chemistry, 132: 943-947.

Patra, J. K., N. K. Dhal and H. N. Thatoi (2011). *In vitro* bioactivity and phytochemical screening of *Suaeda maritima* (Dumort): A mangrove associate from Bhitarkanika, India. Asian Pacific Journal of Tropical Medicine, 4: 727-734.

Peter, M., J. Nakagawa, M. Doree, J. C. Labbe and E. A. Nigg (1990). *In vitro* disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. Cell, 61: 591-602.

Rasool, H. B. (2012). Medicinal plants (importance and uses). Pharmaceut Anal. Acta, 3: e139.

Shangguan, W. J., H. Li and Y. H. Zhang (2014). Induction of G2/M phase cell cycle arrest and apoptosis by ginsenoside Rf in human osteosarcoma MG-63 cells through the mitochondrial pathway. Oncology Reports, 31: 305-313.

Sharma, J. V. C., G. Pitchaiah, D. Satyavati, J. V. Rao and H. Sanjay, (2011). *In vitro* anticancer activity of methanolic extract of roots of *Glochidion zeylanicum* (Gaertn.). IJRPBS, 2: 760-764.

Sleiman, R. J. and B. W. Stewart (2000). Early caspase activation in leukemic cells subject to etoposide induced G2-M arrest: evidence of commitment to apoptosis rather than mitotic cell death. Clinical Cancer Research, 6: 3756-3765.

Stewart, Z. A., M. D. Westfall and J. A. Pietenpol (2003). Cell-cycle dysregulation and anticancer therapy. Trends in Pharmacological Sciences, 24: 139-145.

Täckholm, V. and L. Boulos (1977). Additions and corrections to the second edition of students' flora of Egypt. Publ. Cairo Univ. Herb., 7-8: 211-218.

WHO, World Health Organization (2018). Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018. Geneva, Switzerland, 12 September 2018.

Wu, Z. Z., C. M. Chien, S. H. Yang, Y. H. Lin, X. W. Hu, Y. J. Lu, M. J. Wu and S. R. Lin (2006). Induction of G2/M phase arrest and apoptosis by a novel enediyne derivative, THDA, in chronic myeloid leukemia (K562) cells. Mol. Cell Biochem., 292: 99-105.

Table (1): Forward and reverse primers sequence for *Bcl-2, caspase 3, p53, CycB1, CDK1,* and *β-actin* genes.

Gene	Forward primer (5′ ------ 3′)	Reverse primer (5′ ------ 3′)
Bcl2	AGGAAGTGAACATTTCGGTGAC	GCTCAGTTCCAGGACCAGGC
Caspase 3	TTAATAAAGGTATCCATGGAGAACACT	TTAGTGATAAAAATAGAGTTCTTTTGTGAG
p53	TAACAGTTCCTGCATGGGCGGC	AGGACAGGCACAAACACGCACC
CycB1	CTCCTGTCTGGTGGGAGGA	CTGATCCAGAATAACACCTGA
CDK1	TTTTCAGAGCTTTGGGCACT	CCATTTTGCCAGAAATTCGT
β-actin	CACCAACTGGGACGACAT	ACAGCCTGGATAGCAACG

Table (2): Relative expression of *cycB1* and *CDK1* genes in HepG2 cells following treatments with *Z. album* using qRT-PCR.

Group	Fold change mean
Control (untreated) HepG2 cells	1.00 ± 0.04
cycB1 gene	0.19 ± 0.01
Control (untreated) HepG2 cells	1.00 ± 0.05
CDK1 gene	0.11 ± 0.01

Table (3): Relative expression of *cycB1* and *CDK1* genes in HepG2 cells following treatments with *S. palaestina* using qRT-PCR.

Group	Fold change mean
Control (untreated) HepG2 cells	1.00 ± 0.04
cycB1 gene	0.48 ± 0.03
Control (untreated) HepG2 cells	1.00 ± 0.05
CDK1 gene	0.36 ± 0.04

Table (4): Relative expression levels of *P53, Bcl-2,* and *Caspase 3* genes in HepG2 cells following treatments with *Z. album.*

Group	Fold change mean
Control (untreated) HepG2 cells	1.00 ± 0.08
P53 gene	1.78 ± 0.09
Control (untreated) HepG2 cells	1.00 ± 0.06
Bcl-2 gene	0.24 ± 0.02
Control (untreated) HepG2 cells	1.00 ± 0.09
Caspase 3 gene	2.08 ± 0.14

Table (5): Relative expression levels of *P53*, *Bcl-2*, and *Caspase 3* genes in HepG2 cells following treatments with *S. palaestina.*

Group	Fold change mean
Control (untreated) HepG2 cells	1.00 ± 0.08
P53 gene	2.87 ± 0.12
Control (untreated) HepG2 cells	1.00 ± 0.06
Bcl-2 gene	0.55 ± 0.04
Control (untreated) HepG2 cells	1.00 ± 0.09
Caspase 3 gene	5.46 ± 0.32

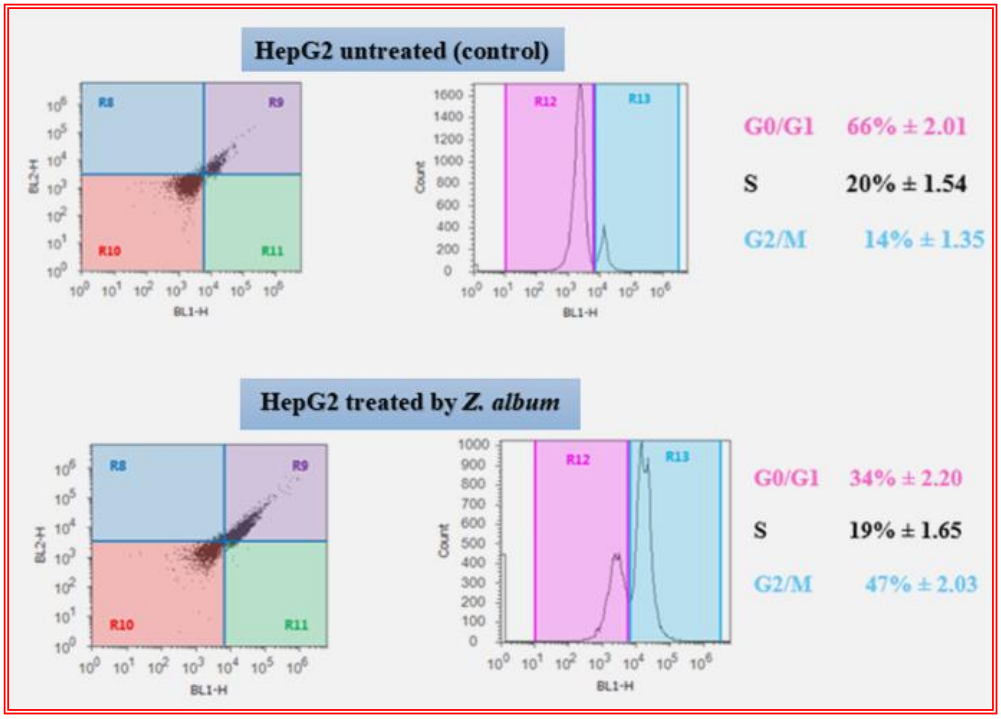

Fig. (1): Effect of *Z. album* on the G2/M cell cycle arrest. Liver cancer cells (HepG2) were treated with *Z. album* at the concentration of 27.74 μg/ml in order to check the cell cycle distribution, then analyzed by flow cytometry.

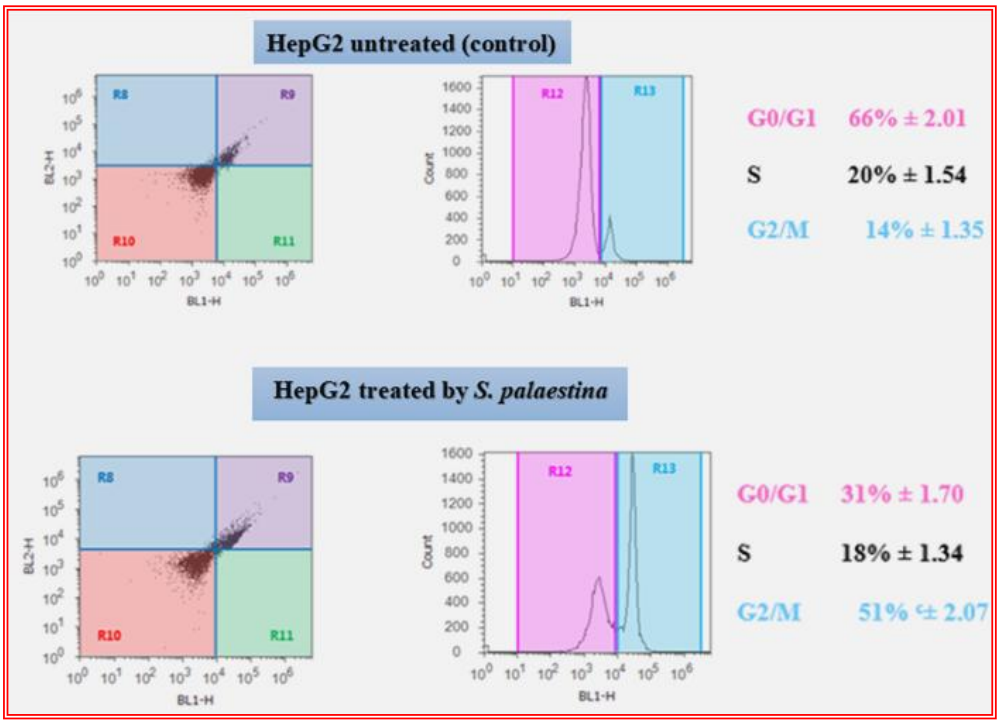

Fig. (2): Effect of *S. palaestina* on the G2/M cell cycle arrest. Liver cancer cells (HepG2) were treated with *S. palaestina* at the concentration of 30.76 µg/mL in order to check the cell cycle distribution, then analyzed by flow cytometry.

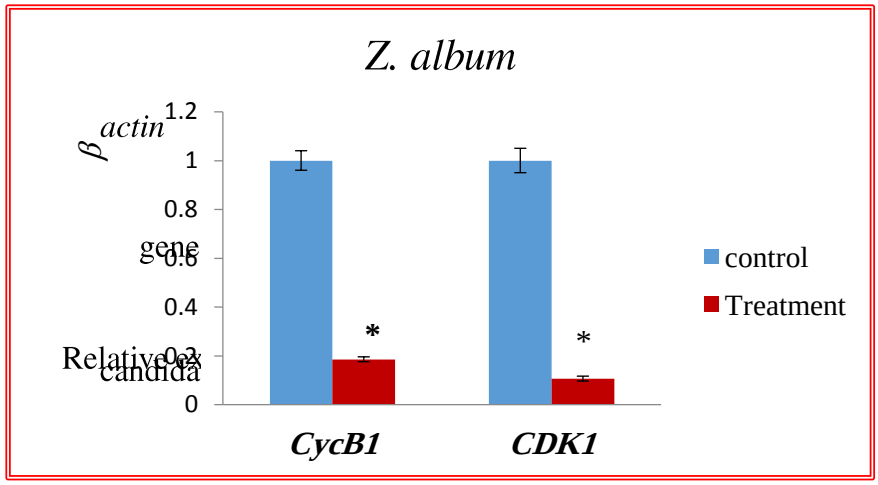

Fig. (3): Effect of *Z. album* on cell cycle regulatory genes at G2/M phase after exposure to 27.74 µg/ml, mRNA expression of *cycB1 and CDK1* genes were assessed by quantitative RT-PCR. *P < 0.05, compared to the control group.

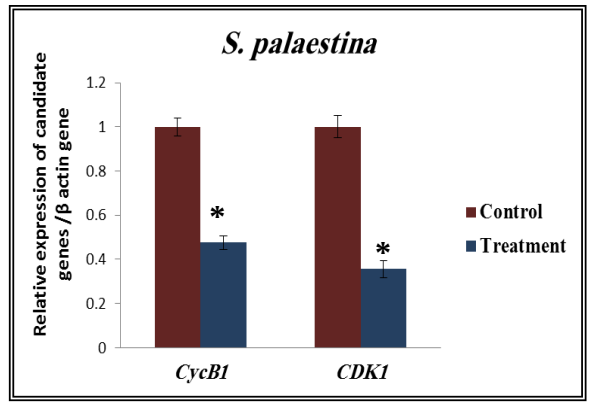

Fig. (4): Effect of *S. palaestina* on cell cycle regulatory genes at G2/M phase after exposure to 30. 76 µg/ml, mRNA expression of *cycB1 and CDK1* was assessed by quantitative RT-PCR. *P < 0.05, compared to the control group.

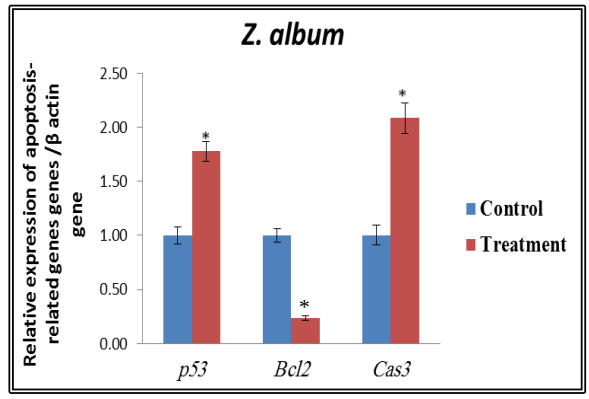

Fig. (5): Effect of *Z. album* on apoptosis-related genes after exposure to 30.76 µg/ml, mRNA expression of *P53, Bcl2, and Caspase 3* genes were assessed by quantitative RT-PCR. *P < 0.05, compared to the control group.

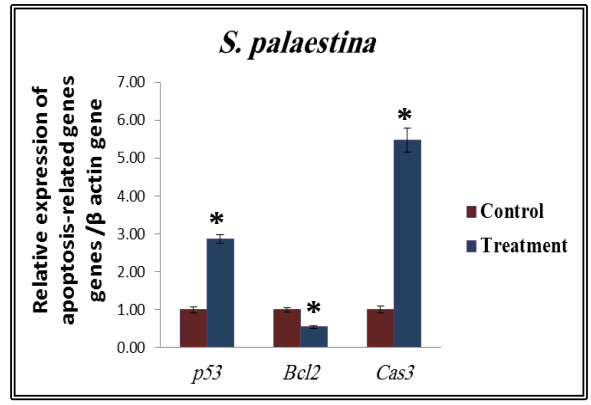

Fig. (6): Effect of *S. palaestina* on apoptosis-related genes after exposure to 30.76 µg/ml, mRNA expression levels of *p53, Bcl2, and Caspase 3* was assessed by quantitative RT-PCR. *P < 0.05, compared to the control group.