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Evaluation of Antiproliferative Activity *In Vivo* of *Atriplex halimus* Extract against Ehrlich Ascites Carcinoma Cells

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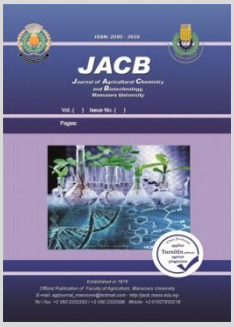

ABSTRACT

Atriplex halimus has demonstrated antiproliferative properties, yet its effects on Ehrlich solid tumor (EST) remain unexplored. This research sought to investigate the therapeutic effect of *A. halimus* ethanolic extract on EST in mice and elucidate its underlying mechanisms of action. To achieve our goal, Swiss albino mice were used to induce EST and divided into four groups negative control, untreated EST, EST treated with 180 mg/kg *A. halimus* extract, and EST treated with 360 mg/kg *A. halimus* extract. Gene expression, protein levels, and DNA damage in tumor tissues were assessed using qRT-PCR, western blot, and comet assay. Our results showed significant tumor size reduction in EST in mice treated with both low and high doses of *A. halimus* crude extract compared to untreated EST mice. Real-time PCR and protein expression analyses revealed that *A. halimus* extract activated apoptotic mechanisms, leading to increase expression of p53, Caspase 3, and cdc2 while downregulating Bcl-2 at the mRNA level in treated EST in mice. Furthermore, the comet assay demonstrated the genotoxic potential of *A. halimus* on solid tumor cells.Our findings show that *A. halimus* may be a promising natural source of anti-proliferative agent for cancer. However, further clinical trials are necessary to prove its effectiveness and safety for therapeutic use. This research provides valuable insights into the molecular mechanisms underlying the antitumor effects of *A. halimus* and highlights its potential in cancer treatment strategies.

Keywords: *Atriplex halimus*, Ehrlich Ascites Carcinoma Cells, Ehrlich solid tumor.

INTRODUCTION

Cancer remains a primary cause of illness and death across the globe. According to WHO 2018, It is estimated that the global incidence of cancer had become 18.1 million new cases in 2018, which led to 9.6 million fatalities. Developing novel and effective therapeutic strategies is necessary (Sung *et al.*, 2021). In recent years, there has been growing interest in medicinal plants as potential sources of anticancer agents, due to their diverse bioactive constituents and generally lower toxicity profiles compared to synthetic drugs (Dar *et al*., 2017, Newman and Cragg, 2020).

Atriplex halimus L. belongs to *Amaranthaceae* family (formerly Chenopodiaceae). The plant occurs naturally across the Mediterranean, Sinai Peninsula, Arabian Peninsula, and East Africa, according to (Boules, 1999, Walker *et al.*, 2014). Traditionally used in folk medicine for various ailments, *A. halimus* has recently gained attention in the scientific community for its potential medicinal properties, including anti-inflammatory and antioxidant activities (Khaldi *et al.*, 2015, Alhamadani *et al*., 2023). Previous investigations have suggested that *A. halimus* exhibits antiproliferative effects, and highly cytotoxic effects on MCF-7 and HepG2 cell lines (Boulaaba *et al.*, 2013, Al-Senosy *et al*., 2018 & Alhamadani *et al.*, 2023). However, its impact on solid tumors in vivo, particularly the Ehrlich solid tumor (EST) model, has not been thoroughly investigated.

The Ehrlich solid tumor model, which originated from a spontaneous mice mammary adenocarcinoma, is widely used in cancer research due to its rapid growth and reproducibility (Ozaslan *et al.*, 2011). This model provides valuable insights into tumor progression and serves as a platform for evaluating potential anticancer agents (El-Magd *et al*., 2017& Elsayed *et al*., 2020). To promote tumor growth, EAC induces a local inflammatory reaction with accumulated vascular permeability, resulting in severe oedema, increasing ascitic fluid generation, and cellular migration (Mutar *et al*., 2020). Without medication, the animal may perish 17-18 days after EAC injection due to the rapid proliferation of EAC cells, which fill the peritoneal cavity with ascitic fluid (Magdy *et al*., 2020).

Programmed cell death, or apoptosis, is a critical factor in the progression and treatment of cancer. The tumor suppressor p53, the anti-apoptotic protein Bcl-2, and the executioner enzyme caspase-3 are among the primary regulators of apoptosis. (Mahani *et al.*, 2015, Pistritto *et al.*, 2016). Additionally, cell cycle regulators such as cdc2 (cell division cycle 2) are important in controlling cell proliferation (Malumbres and Barbacid 2009). Understanding how potential anticancer agents modulate these molecular pathways is essential for elucidating their mechanisms of action.

The Comet test was used to identify potential DNA fragmentation in tumor cells following various therapies (Sharawi 2020).

The current research aimed to evaluate the therapeutic effect of *A. halimus* ethanolic extract on Ehrlich solid tumor in mice and to elucidate its underlying

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mechanisms of action. By examining the effects of *A. halimus* on tumor growth, apoptosis-related gene expression, and DNA damage, this research seeks to provide comprehensive insights into its anticancer properties. The results of this study may have the potential to enhance the development of innovative, plant-based cancer treatment strategies and provide a more comprehensive understanding of the molecular pathways that are involved in the antitumor effects of *A. halimus*.

MATERIALS AND METHODS

Plant Collection and Extract Preparation

The *Atriplex halimus* plant was gathered from Wadi Gharandal in South Sinai, Egypt, by Dr. Mohamed Abd El-Maboud of the Desert Research Center, Egypt. It was subsequently identified in the Medical and Aromatic Laboratories of the Horticulture Department at the Faculty of Agriculture, Ain Shams University. The ethanolic extract of *A. halimus* was prepared according to Al-Senosy *et al.,* 2018.

Animals and Experimental Design

Male Swiss albino mice (6-8 weeks old, weighing 20-25g) had been obtained from The National Cancer Institute at Cairo University. The animals were kept in polypropylene cages (7 mice/cage) under typical laboratory circumstances (25 to 27°C, 12h light/dark cycle, humidity of 55 to 57%) with free access to conventional pellet food and water ad libitum.

Male Swiss albino genotype mice (6-8 weeks old, weighing 20-25g) had been obtained from The National Cancer Institute at Cairo University. The animals were kept in polypropylene cages (7 mice/cage) under typical laboratory circumstances (25 to 27°C, 12h light/dark cycle, humidity of 55 to 57%) with free access to conventional pellet food and water ad libitum.

To sustain Ehrlich ascites carcinoma (EAC) cells in mice, 1×106 cells were inoculated intraperitoneally weekly. On day zero (D0), the Ehrlich ascites carcinoma (EAC) was intramuscularly injected with just one dose of 0.2 mL EAC (2×106 cells) in the femoral region to induce the Ehrlich solid tumor (EST) (Fahim *et al*., 1997).

Twenty-eight mice were randomly split into four groups (n=7 per group):

- Group I: Negative control (C) (no tumor, no treatment)
- Group II: EST control (EST) (tumor-bearing, no treatment)
- Group III: EST + 180 mg/kg A. halimus extract.
- Group IV: EST + 360 mg/kg A. halimus extract.

The *Atriplex halimus* ethanolic extract was administered day after day by intratumoral injections three times a week for two weeks, starting 24 hours after solid tumor induction (tumor formation). Control groups were given an identical volume of the vehicle (distilled H2O) (Li *et al*., 2007).

after each treatment of the experiment on 2nd, 4th, 6th, 8th,10th, and 12th days samples were taken and the mice were killed via beheaded under ether anesthesia. The tumors were subsequently separated into three distinct portions: one part was conserved in 10% formalin for histopathological analysis, another part was stored at -80 degrees Celsius for RT-PCR and western blotting, and the last section was preserved at -20 degrees Celsius for comet assay procedures.

Tumor Reduction Rate

The formula used to express the tumor reduction rate is adhered to: Tumor reduction rate (%) = (Average of tumor weight in Control group – Average of tumor weight in Treated group) / (Average of tumor weight in Control group) × 100.

Histopathological Inspection

After fixation, the tumors were covered in paraffin and sectioned into 4 µm slices. These slices were dyed with Hematoxylin and Eosin and inspected using a light microscope.

Table 1. Primer Sequence for Tumor and Housekeeping Genes

Genes	Primer Sequence	References
p53	F: 5' CCCAGGTCCAGATGAAG-3' R: 5' CAGACGGAAACCGTAGC-3'	(Sharawi, 2020)
Bcl2	F: 5' CATGCCAAGAGGGAAACACCAGAA 3' R: 5' GTGCTTTGCATTCTTGGATGAGGG 3'	(Sharawi, 2020)
caspase3	F: 5' TTCATTATTCAGGCCTGCCGAGG 3' R: 5' TTCTGACAGGCCATGTCATCCTCA 3'	(Sharawi, 2020)
Cdc2	F: 5'AAGTGTGGCCAGAAGTCGAG3' R: 5' TCGTCCAGGTTCTTGACGTG 3'	(Sharawi, 2020)
β-actin	F: 5' CTGTCCCTGTATGCCTCTG 3' R: 5' ATGTCACGCACGATTTCC3'	(Sharawi, 2020)

Analysis of Gene Expression in Apoptosis-Regulatory Genes

The Gene JET RNA Purification Kit (Thermo Scientific, # K0731, USA) was employed to extract total RNA from tumor tissues following the company's guidelines. RNA purity and concentration were evaluated spectrophotometrically. cDNA was synthesized via a reverse transcription kit (Applied Biosystems) following the company's protocol.

Real-time PCR was conducted with SYBR Green PCR Master Mix (Applied Biosystems) on Step One Plus Real-Time PCR System. Table 1 contains the primers for tumor genes and housekeeping genes (Sharawi, 2020). qRT-PCR mix and condition were carried out according to Sharawi, 2020 The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) was utilized to assess the relative gene expression compared to housekeeping gene (β-actin) serving as the internal control.

Protein Expression Analysis (Western Blot)

A methodology defined by Sharawi, 2020 was employed to evaluate alterations in the expression of p53, caspase 3, and Bcl2 proteins after various treatments. Western blot was subsequently performed.

DNA Damage Assessment (Comet Assay)

The comet test was conducted as previously explained (Badawy *et al.,* 2018, El-Magd *et al.,* 2019, Effat *et al.,* 2023) to determine the effect of various treatments on DNA fragmentation in tumor tissues. The DNA samples were dyed using GelRed dye, resulting in a red color under a fluorescence microscope. The Komet 5 image analysis program (Kinetic Imaging, Liverpool, UK) was used to assess DNA damage metrics such as comet tail length, percentage of destroyed DNA, and tail moment. Each sample had around 50 to 100 cells examined.

Statistical analysis

The means ± standard error (SE) were used to describe all results. The statistical significance was assessed using SPSS 18.0 software and a one-way ANOVA. Values

were deemed statistically significant when P≤0.05. Tukey's Honestly Significant Difference (HSD) test was employed to conduct a comparison of means.

RESULTS AND DISCUSSION

Results

Effect of *Atriplex halimus* on tumor growth

Ehrlich solid tumor mice (EST) model

The Ehrlich solid tumor (EST) mice model was generated via intramuscular injection of EAC cells into mice using 2 doses (180, 360 mg/kg) day after day for 12 days to assess the antitumor properties of *Atriplex halimus* extract.

The tumor weight readings were taken concurrently with dose administration. The results showed an increase in tumor size in the EST group, while treatment with *Atriplex halimus* extract significantly reduced tumor size in EST-bearing mice. Compared to the untreated EST group, mice receiving 180 mg/kg and 360 mg/kg of *A. halimus* extract showed 45% and 63% reduction in tumor volume by day 12 ($p<0.01$). Moreover, the high-dose treatment showed more reduction with significant differences (Fig 1 and 2).

Effect of *Atriplex Halimus* on Tumor Cell Histology

Anaplastic cells with mitotic figures were observed in the histological inspection of tumor masses in the EST group, and there was no proof of cell death or apoptosis (Fig. 3A&B), where the yellow arrow showed the cell division; Fig. (3A) illustrated cell in metaphase and Fig. (3B) demonstrated cell in telophase. Nevertheless, both treatment groups exhibited distinct characteristics of extensive necrosis, such as nucleus atrophy, a disintegrating and structureless red staining region, and apoptosis (Fig. 3C) in conjunction with necrosis (Fig. 3D).

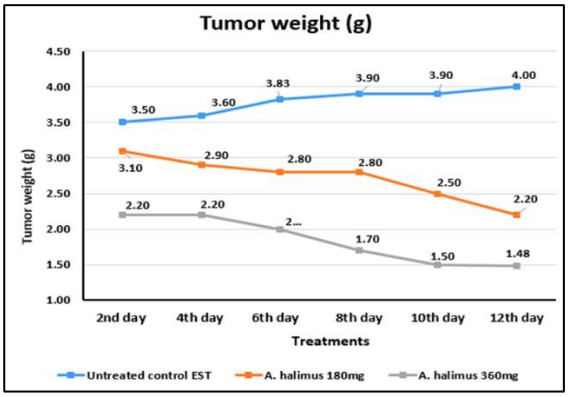

Fig. 1. Impact of *Atriplex halimus* extract (two doses) on tumor growth of EST mice model.

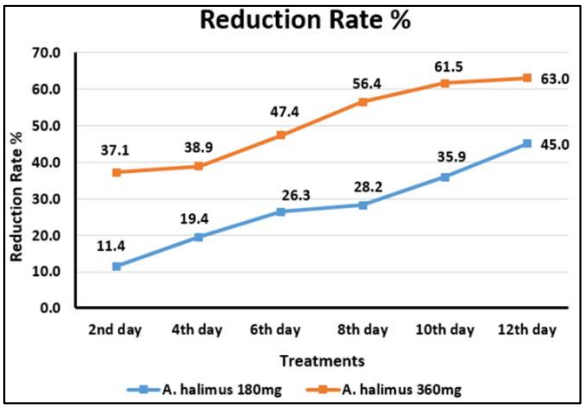

Fig. 2. Diagram for the tumor inhibition % of *Atriplex halimus* extract (180 mg and 360 mg/kg) on tumor growth of EST mice model.

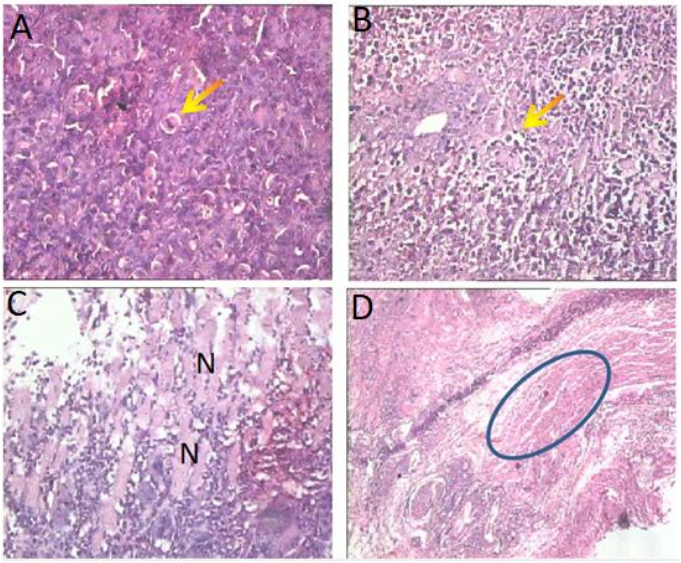

Fig. 3. Light micrographs of the solid tumor tissue sections have been dyed with H&E. In the control group EAC (A and B) the tumor masses were formed of anaplastic with mitotic figures (yellow arrow showed metaphace (A) and Telophase (B)) (40×). C and D) Sections of tumor tissues in mice treated with *Atriplex halimus* (180 mg/kg and 360 mg/kg) illustrated massive necrosis and apoptotic cells. (C and D) illustrated the structural heterogeneity of the tumor, which is characterized by the interspacing of viable areas by necrotic zones that have been infiltrated (N and circle).

Impact of *Atriplex Halimus* Crude Extract Altered the Expression Level of Cell Cycle Regulatory Genes and Apoptotic Genes

Based on the reduction rate of solid tumor tissues, we aim to verify whether the antiproliferative effect of *A. halimus* is linked to the expression levels of genes that regulate the cell cycle and apoptosis. Therefore, quantitative Real-time PCR was carried out and samples were taken on the 2nd, 4th, 6th, 8th,10th, and 12th days, all samples exhibited

melting curve peaks at approximately the same Tm for the target sequence of each gene tested.

In general, the qRT-PCR analysis revealed significant alterations in the expression of apoptosis and cell cycle-related genes. Significant increases in gene expression of *p53*, *caspase3*, and *cdc2* genes were observed after each treatment compared to the previous treatment when compared to the Ehrlich solid tumor (EST) experiment. While the gene expression of *Bcl2* was decreased, showed in Figs (4, 5, 6, and 7), respectively.

On day 12, the expression of *p53* was significantly up-regulated in tumor tissues, showing a 6.79-fold in the group treated with a low dose and a 13.17-fold in the group treated with a high dose, in comparison to the untreated EST group (Fig. 4). The other apoptotic gene (*caspase3*) also showed increased expression after treatment with *A. halimus*, as the gene expression was 1.99 times at the low dose and 3.90 times at the high dose (Fig. 5). Conversely, *bcl2* significantly decreased in expression level (3.47-fold with the low dose and 1.63-fold with the high dose), as compared to the untreated EST (8.69 fold) (Fig. 6).

Additionally, treatment of *A. halimus* at doses of 180 mg/kg and 360 mg/kg, resulted in a significantly increased The cell cycle regulator *cdc2*, exhibiting gene expression increases of 2.48-fold and 3.09-fold, respectively, as compared to the untreated EST, $p<0.05$ (Fig. 7).

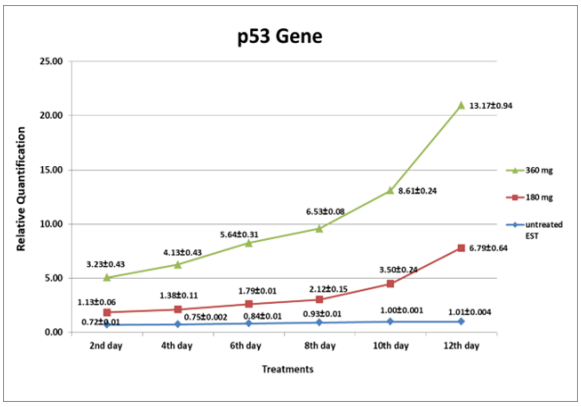

Fig. 4. The impact of *A. halimus* on the *p53* gene expression after exposure to 180 mg/kg and 360 mg/kg was assessed in comparison to the untreated EST group using qRT-PCR.

Data showed as mean ±SE.

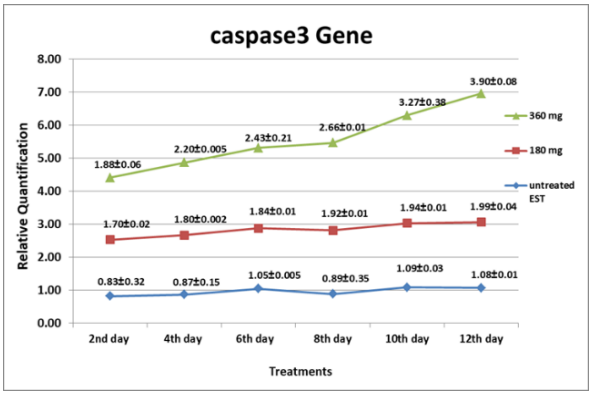

Fig. 5. The effect of *A. halimus* on the *caspase3* gene after exposure to 180 mg/kg and 360 mg/kg was assessed in comparison to the untreated EST group using qRT-PCR.

Data showed as mean ±SE.

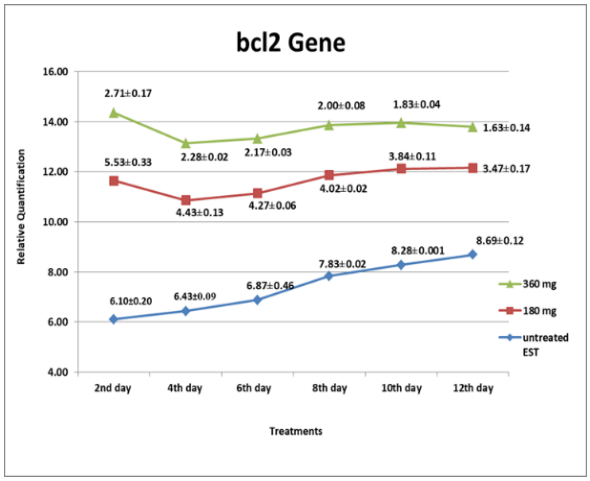

Fig. 6. The effect of *A. halimus* on the *Bcl2* gene after exposure to 180 mg/kg and 360 mg/kg was assessed in comparison to the untreated EST group using qRT-PCR.

Data showed as mean ±SE.

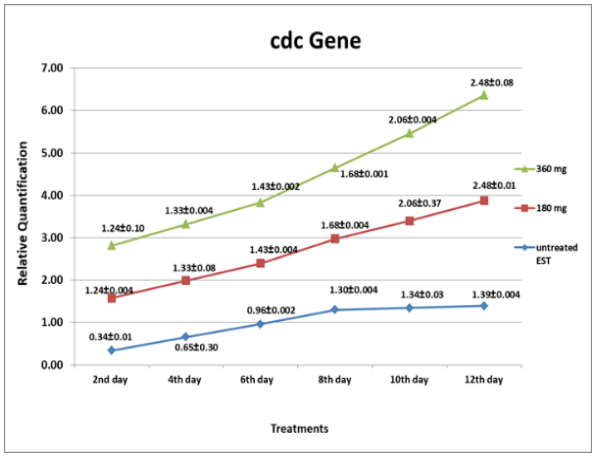

Fig. 7. The effect of *A. halimus* on the *cdc2* gene after exposure to 180 mg/kg and 360 mg/kg was assessed in comparison to the untreated EST group using qRT-PCR.

Data showed as mean ±SE.

These results revealed that *Atriplex halimus* enhanced the activity of the tumor inhibitory gene (*p53*), which is essential for cell cycle control, the repair mechanism, and apoptosis (programmed cell death). As well as they activated the expression of *caspase3* gene, which is a clear indicator of cell death programs. Moreover, the expression of *cdc2* was elevated, while the expression of anti-apoptotic *bcl2* was reduced, which enhances the survival of altered cells and assists in the development of cancer (Aswathi, *et al*. 2024). All the above indicates that *Atriplex halimus* plant ethanolic extract suppressed the cellular proliferation of EST tissues via induction of the apoptotic pathway.

To decrease the detrimental consequences of reactive oxygen species (ROS), the *p53*-enhanced antioxidant mechanism by elevated GST (glutathione S-transferase) levels was implemented. Assessment of *bcl2*, *caspase3*, and *p53* expression is a prevalent method employed to analyze apoptosis in response to treatment with related compounds. For instance, HepG2 cells have been reported to undergo either downregulation of *bcl2* or upregulation of *p53* and caspase 3 following the induction

of apoptosis by other compounds (Aswathi, *et al.* 2024). This finding is consistent with the results of Al-Senosy *et al.* (2018) and Elbouzidi *et al.* (2022), who observed that *Atriplex halimus* inhibited the proliferation of HepG2 cells by activating apoptosis. Simultaneously, the antitumor effect of *A. halimus* -induced cancer cell mortality may be a result of *caspase3* activation, as indicated by the current study (Elbouzidi *et al.*, 2022).

These results agree with Alhamadani, *et al.*, (2023), who noted that extract of *A. halimus* exhibits anticancer properties and induction of apoptosis on HepG2 cells. Furthermore, the *A. halimus* extract has shown considerable antioxidant activity and confirmed that *A. halimus* possesses the potential for incorporation into medicinal formulations, owing to the presence of a blend of kaempferol, quercetin, and other bioactive compounds.

In the primary cell culture of human liver carcinoma selected for this investigation, *A. halimus* extracts demonstrate anticancer effects, which can be enhanced by morphological changes that promote the induction of apoptosis. Additionally, the extract of *A. halimus* demonstrated significant antioxidant properties as evidenced by the measurements of catalase and superoxide dismutase enzymes. This plant extract has the capability to be incorporated into medicinal products. As a result of the presence of a mixure of kaempferol and quercetin, as well as other compounds that are bioactive.

Impact of *A. halimus* on apoptosis-related proteins

Western blot examination was carried out to assess altrations in the levels of *p53, bcl2* and *caspase3* proteins in the EST mice after treatment by *A. halimus* in comparison with negative control and untreated EST. The expression level of *p53* and *caspase3* proteins were remarkably upregulated, and the expression of *bcl2* was downregulated after *A. halimus* (180mg/ kg and 360mg/ kg) treatment in comparison with the negative control and untreated EST groups (Table 2 and Fig. 8). In all cases, groups treated with high doses showed a significantly elevated *p53* and *caspase3* expression than the low dose-treated group. Nevertheless, there was no discernible distinction in *bcl2* expression among the two doses under investigation.

The expression level of *p53*, and *Caspase 3* proteins were significantly up regulated with relative densities of 4.36±0.25 and 5.18±0.32 for *p53*, moreover, 2.58±0.11 and 5.60±0.37 for *caspase3* after treated with *A. halimus* (180mg/ kg and 360mg/ kg), respectively, in comparison with untreated EST group 0.96±0.07 for *p53* and 0.90±0.07 for *caspase3* gene. While the expression protein of *bcl2* was down-regulated after treatment by *A. halimus* with relative densities of 0.34±0.02 in 180mg/kg and 0.19±0.01 in 360mg/kg treatments, respectively. These results are recorded in Table (2).

These results confirmed that the *A. halimus* extract encourages apoptosis by activating *p53* and *caspase3*, and inactivating *bcl2*, as confirmed by gene expression of RNA and protein investigations. These data collectively indicate that *A. halimus* may be a viable candidate for the production of anticancer drugs.

Table 2. Band quantification of p53, caspase 3 and *Bcl*-2 proteins expression after treatment by *A. halimus* extract with two concentrations.

Group	***P53* protein expression**	***Caspase 3* protein expression**	***Bcl-2* protein expression**
	Relative density	**Relative density**	**Relative density**
Untreated EST	0.96± 0.07	0.90 ±0.07	0.99 ±0.08
A. halimus (180mg/kg)	4.36 ± 0.25	2.58 ± 0.11	0.34 ±0.02
A. halimus (360mg/kg)	5.18 ± 0.32	5.60 ± 0.37	0.19 ±0.01

Different superscript letters in the same column of tail length showed significant differences at P< 0.05.

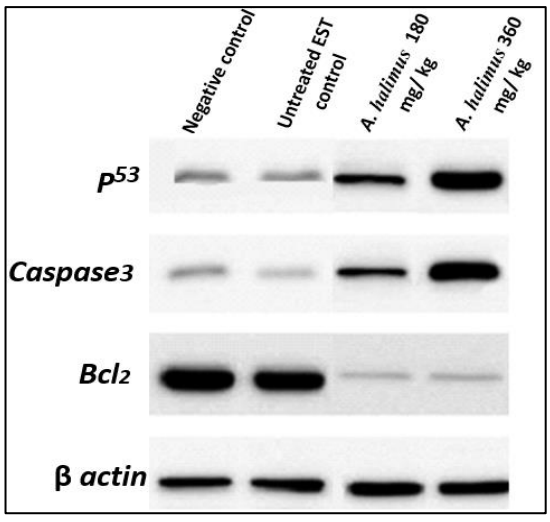

Fig. 8. Western blot profiles illustrated the impact of *A. halimus* extract treatment on protein expression of *p53, caspase3,* and *bcl2* in EST tumors in comparison to (untreated) EST and negative control groups. The internal control protein was *β actin*.

The impact *of A. halimus on* DNA fragmentation

In this study, the assessment of the DNA fragmentation (using the single cell gel-electrophoresis (comet assay) in an Ehrlich solid tumor cell exposed to the *A. halimus* was determined. In comparison to the untreated EST group, the *A. halimus* ethanolic extract produced significant DNA fragmentation in EST tissues, as evidenced by an increase in tailed DNA %, untailed DNA %, tail DNA %, and tail moment (Table 3 and Fig. 9) in the low and high doses of *A. halimus.*

Our results revealed that *A. halimus* induced substantial DNA fragmentation which was evident from the appearance of comet length 6.72±0.34 in 180mg/kg and 8.82±0.46 µm in 360mg/kg which was much more than that in the untreated EST group (2.24±0.13µm). The high doses of *A. halimus* inflicted significantly higher fragmentation effect on DNA of EST cells than low doses, in tail DNA percentage was 2% in EST, while, 17% in low dose (180mg/kg) and 26% in high dose (360mg/kg), These results noted in Table (3). The treated groups showed a significant increase in the tail length of the comet (as an indicator for DNA fragmentation).

These results indicate that *A. halimus* ethanolic extract possesses antitumor potential, evidenced by its ability to induce apoptosis in EST cells through severe DNA fragmentation compared to the negative control.

Table 3. Detection of DNA fragmentation by the comet assay

Group	Tailed DNA (%)	Untailed DNA (%)	Tails length (µm)	Tail DNA (%)	Tail moment
EST	2	98	2.24±0.13 [c]	2.1	3.96
A. halimus (180 mg)	17	83	6.72±0.34 [b]	4.81	32.32
A. halimus (360mg)	26	74	8.82±0.46 [a]	7.45	65.71

A significant difference was observed at P< 0.05 when various superscript letters were used in the same tail length column.

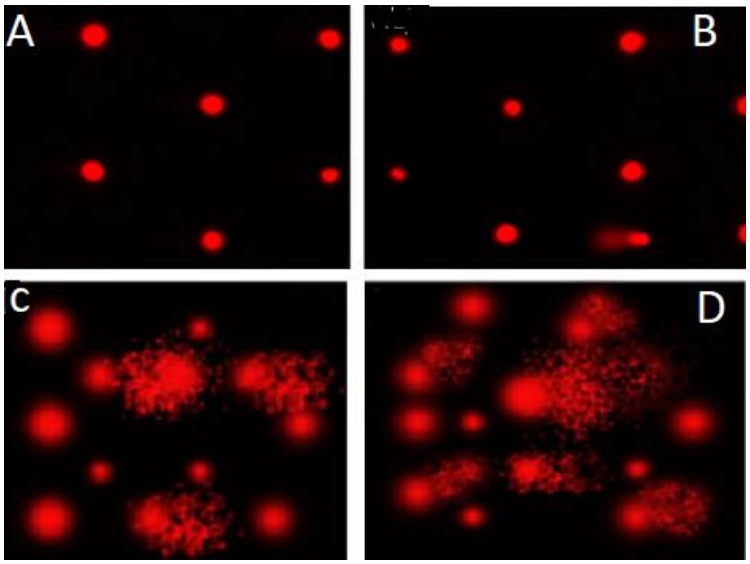

Fig. 9. Comet images illustrated DNA fragmentation in Ehrlich solid tumor (EST) cells. (A) represents DNA strand in negative control mice, (B) represents DNA strand breaks of untreated EST, (C) EST after treating with 180mg/kg of *A. halimus* extract, and (D) represents EST after the exposure to *A. halimus* extract.

Discussion

The present study demonstrates the potent antitumor effects of *Atriplex halimus* extract on Ehrlich solid tumor in mice, providing insights into its potential mechanisms of action. The observed tumor growth inhibition aligns with previous studies on other medicinal plants, suggesting that *A. halimus* may contain bioactive compounds with antiproliferative properties (Al-Senosy *et al.*, 2018, Attia *et al.*, 2022).

The purpose of this investigation was to assess the potential anti-mutagenic and anti-carcinogenic impacts of the *A. halimus*, on Ehrlich solid tumors in mice. As far as we are aware, this research may be the first to report that *A. halimus* has an anti-cancer activity against EST. This overall conclusion was derived from the findings that treating with *A. halimus* led to the following: 1) a reduction in the volume of tumor; 2) an elevated level in tissue necrosis and apoptosis (as proven by histological inspection); 3) downregulation of the *bcl2* gene and protein; 4) upregulation of the *p53* and *caspase3* gene and protein, as well as the DNA fragmentation.

Suppression of apoptosis is considered an important initial factor in tumorigenesis development, which allows uncontrollable proliferation of the cell. Consequently, the induction of apoptosis serves as the primary mechanism of mode of action for most anti-cancer agents (Chaudhry *et al.*, 2022).

Several studies have concentrated on the critical function of *p53* in the equilibrium among proliferation and apoptosis (Polager and Ginsberg, 2009). The *p53* gene is essential for the suppression of the G2/M transition during the G2 checkpoint, which ensures that any potential deleterious effects are prevented after that point. Additionally, it controls the equilibrium between the antiapoptotic *bcl2* gene and the proapoptotic *bax* gene via its gene expression (Leu *et al.*, 2004). The *bcl2* genes are essential for the regulation of the mitochondrial mechanism of apoptosis (Dewson and Kluck, 2010). This pathway is composed of pro-apoptosis genes (*puma, bax, bim, bid, noxa*), anti-apoptosis genes (*bcl2, bcl-xl*), and one of the permeability of mitochondrial transition pores (Chen and Lesnefsky, 2011). *bcl2* can maintain the permeability of the mitochondria transition and prevent the excretion of cytochrome c, which would otherwise deactivate caspase (Li *et al.*, 1997). Caspases are significant regulators of programmed cell death (apoptosis). *caspase3* is a death protease that is frequently activated and is responsible for the precise cleavage of numerous critical cellular proteins. The *caspase3* is crucial for particular steps that are linked to the disruption of the cell and the formation of apoptotic bodies. However, it may also operate prior to or during this phase when cell viability is necessary (Porter and Jänicke, 1999).

Declaration

Ain Shams University Committee on Experimental Animal Care and Studies Ethics, Agriculture Sector Committee, authorized all studies involving the use of animals (permission No. 15-2024-02).

All procedures involving animals were executed following the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The animals' welfare was prioritized throughout the study, ensuring minimal distress and pain.

We affirm that all personnel involved in the study were trained in the proper handling and care of animals and that all efforts were made to decrease the number of animals

used following the rules of the 3Rs (Replacement, Reduction, and Refinement).

By adhering to these ethical standards, we aim to ensure the integrity of our research and the humane treatment of all animals involved.

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REFERENCES

Akhter N., Dar S.A., Haque S., Wahid M., Jawed A., Akhtar M.S., Alharbi R.A., Sindi A.A.A., Alruwetei A., Choudhry H.M. Z., Ahmad A. (2021). Crosstalk of Cyclin-dependent kinase inhibitor 1A (CDKN1A) gene polymorphism with p53 and CCND1 polymorphism in breast cancer. European Review for Medical and Pharmacological Sciences. 25: 4258-4273

Abdel-Gawad, E.I., Hassan, A.I., and S.A. Awwad (2016). Efficiency of calcium phosphate composite nanoparticles in targeting Ehrlich carcinoma cells transplanted in mice. Journal of Advanced Research, 7(1): 143-154.

Alhamadani, A. H., Jabbar, E. A. K., & Al-Waheeb, A. N. (2023). Study on The Bioactive Components of *Atriplex halimus* L. and Evaluation of Their Anticancer Effect on Liver Cancer Cells. The Egyptian Journal of Hospital Medicine, 90, 928-936.

Al-Senosy N., K., Abou-Eisha A., and Ahmad E.S. (2018). Cytotoxic effects of *Atriplex halimus* extract on human cancer cell lines. Bioscience Research 15(3):1718-1728.

Aswathi R. k., Arumugam S, Muninathan N, Baskar K. Deepthi S, Dinesh Roy D. (2024). P53 Gene as a Promising Biomarker and Potential Target for the Early Diagnosis of Reproductive Cancers. Cureus 16(5): e60125. Doi: 10.7759/cureus.60125.

Attia AA, Salama AF, Eldiasty JG, Mosallam SAE-R, El-Naggar SA, El-Magd MA, Nasser HM, Elmetwalli A (2022). Amygdalin potentiates the anti-cancer effect of Sorafenib on Ehrlich ascites carcinoma and ameliorates the associated liver damage. Sci Rep 12:6494. doi.org/10.1038/s41598-022-10517-0.

Boulaaba M., Mkadmini K, Tsolmon S., Han J., Smaoui A., Kawada K., Ksouri R., Isoda H., Abdelly C. (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. Evidence-Based Complementary and Alternative Medicine. Article ID 529375, 11 pages. http://dx.doi.org/10.1155/2013/529375

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

Chaudhry G.E.S., Akim A., Sung Y.Y. and Sifzizul T.M.T. (2022). Cancer and apoptosis: The apoptotic activity of plant and marine natural products and their potential as targeted cancer therapeutics. Frontiers in Pharmacology. 13:842376. doi: 10.3389/fphar.842376.

Chen, Q. and E.J. Lesnefsky (2011). Blockade of electron transport during ischemia preserves Bcl-2 and inhibits opening of the mitochondrial permeability transition pore. FEBS Letters, 585(6): 921-926.

Dar, R.A., Shahnawaz, M., and Qazi, P.H. (2017). General overview of medicinal plants: A review. The Journal of Phytopharmacology, 6 (6): 349-351.

Dewson, G.K.R.M. and Kluck R.M. (2010). Bcl-2 family-regulated apoptosis in health and disease. Cell Health and Cytoskeleton, 2: 9- 22.

Dolai, N.; Islam, A. and P.K. Haldar (2016). Methanolic extract of *Anthocephalus cadamba* induces apoptosis in Ehrlich ascites carcinoma cells in experimental mice. Indian Journal of Pharmacology, 48 (4): 445-449.

Effat Saied N, Elmazny GM, El-Helaly RM, Farag RE, Abd El-Wahab K, Abo Hashim E, El-Zehery RR. (2023). Utility of comet assay of DNA damage in the detection of malignant transformation of chronic liver cirrhosis. Scand J Clin Lab Invest. 2023 May;83(3):145-151. doi: 10.1080/00365513.2023.2175327.

Elbouzidi A, Ouassou H, Aherkou M, Kharchoufa L, Meskali N, Baraich A, Mechchate H, Bouhrim M, Idir A, Hano C, Zrouri H, (2022). Addi M. LC-MS/MS Phytochemical Profiling, Antioxidant Activity, and Cytotoxicity of the Ethanolic Extract of *Atriplex halimus* L. against Breast Cancer Cell Lines: Computational Studies and Experimental Validation. Pharmaceuticals (Basel). Sep 16;15(9):1156. doi: 10.3390/ph15091156. PMID: 36145377; PMCID: PMC9503641.

El-Magd MA, Khamis A, Nasr Eldeen SK, Ibrahim WM, Salama AF (2017). Trehalose enhances the antitumor potential of methotrexate against mice bearing Ehrlich ascites carcinoma. Biomed Pharmacother 92:870–878. doi: 10.1016/j.biopha.2017.06.005.

Elsayed SA, Harrypersad S, Sahyon HA, El-Magd MA, Walsby CJ (2020). Ruthenium (II)/(III) DMSO-Based Complexes of 2-Aminophenyl Benzimidazole with In Vitro and In Vivo Anticancer Activity. Molecules 25(18), 4284. doi.org/10.3390/molecules25184284.

Fahim, F.A.; Esmat, A.Y.; Mady, E.A. and M.A. Amin (1997). Serum LDH and ALP isozyme activities in mice bearing solid Ehrlich carcinoma and/or treated with the maximum tolerated dose (MTD) of aloin. Disease Markers, 13 (3): 183-193.

Khaldi A., Amamra D., Tir touil A., Maghdouri N,Belhadj N, (2015). Effects of *Atriplex halimus* Resistant Bacterial strain of Different origins, International Conference on Advances in Agricultural, Biological & Environmental Sciences (AABES2015) July 22-23, London (UK).

Leu, J.J.; Dumont, P.; Hafey, M.; Murphy, M.E. and D.L. George (2004). Mitochondrial *p53* activates *Bak* and causes disruption of a Bak–Mcl1 complex. Nature Cell Biology, 6 (5): 443- 450.

Li J., Li Q., Feng T., Zhang T., Li K., Zhao R., Han Z., Gao D. (2007). Antitumor activity of crude polysaccharides isolated from Solanum nigrum Linne on U14 cervical carcinoma bearing mice. Phytotherapy Research; 21(9):832-840. doi: 10.1002/ptr.2163. PMID: 17486683.

Li, P.; Nijhawan, D., Budihardjo, I., Srinivasula, S.M., Ahmad, M., Alnemri, E.S. and Wang X. (1997). Cytochrome c and dATP-dependent formation of *Apaf-1/caspase-9* complex initiates an apoptotic protease cascade. Cell, 91 (4): 479-489.

Magdy A, Sadaka E, Hanafy N, El-Magd MA, Allahloubi N, El Kemary M (2020). Green tea ameliorates the side effects of the silver nanoparticles treatment of Ehrlich ascites tumor in mice. Mol Cell Toxicol 16:271–282. doi.org/10.1007/s13273-020-00078-6.

Mahani, E.S.; Ebrahimi, B.; Abbasnejad, M.; Rasoulian, B. 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 (2): 165-170.

Malumbres M., Barbacid M. (2009). Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer. 9(3):153-166.

Mutar T.F., Tousson E., Hafez E., Abo Gazia M., Salem S.B. (2020). Ameliorative effects of vitamin B17 on the kidney against Ehrlich ascites carcinoma induced renal toxicity in mice. Environmental Toxicology. 35:528–537. doi: 10.1002/tox.22888.

Newman D.J., Cragg G.M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. J Nat Prod.;83(3):770-803.

Ozaslan M., Karagoz I.D., Kilic I.H., Guldur M.E. (2011). Ehrlich ascites carcinoma. Afr J Biotechnol. 10(13): 2375-2378.

Pistritto G., Trisciuoglio D., Ceci C., Garufi A., D'Orazi G. (2016). Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY).;8(4):603-619.

Polager, S. and D. Ginsberg (2009). *p53* and *E2f*: partners in life and death. Nature Reviews Cancer, 9 (10): 738-748.

Porter, A.G. and Jänicke R.U. (1999). Emerging roles of *caspase-3* in apoptosis. Cell Death and Differentiation, 6 (2): 99- 104.

Sharawi, W. Z., (2020). Therapeutic effect of Arthrocnemum machrostachyum methanolic extract on Ehrlich solid tumor in mice. BMC Complementary

Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A, Bray F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 71 (3): 209-249.

Walker D.J., Lutts S., Sánchez-García M., Correal E. (2014). *Atriplex halimus* L.: Its biology and uses. J Arid Environ. 100-101:111-121.

تقييم النشاط المضاد للانتشار لمستخلص نبات القطف البحرى *Atriplex Halimus* ضد خلايا سرطان الاستسقاء إيرليش في الجسم الحي

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الملخص

لقد أظهر مستخلص نبات القطف البحرى *Atriplex halimus* خصائص مضادة للانتشار، إلا أن تأثيراته على الورم الصلب إيرليش (EST) لا تزال غير مستكشفة. هدف هذا البحث إلى التحقيق في الإمكانات العلاجية لمستخلص نبات القطف الإيثانولي على EST في فئران التجارب وتوضيح آليات عمله الأساسية. لتحقيق هدفنا، تم استخدام 28 فأر من الفئران البيضاء السويسرية لتحفيز EST وتقسيمها إلى أربع مجموعات: مجموعة (مجموعة تحكم سالبة) فئران غير محفز بها EST وغير معالجه ، ومجموعة فئران محفز بها EST وغيرمعالجة ، ومجموعة فئران محفز بها EST ومعالجة بـ 180 مجم / كجم من مستخلص نبات القطف ، ومجموعة فئران محفز بها EST ومعالجة بـ 360 مجم / كجم من مستخلص نبات القطف. تم تقييم التعبير الجيني ومستويات البروتين وتلف الحمض النووي في أنسجة الورم باستخدام تفاعل البلمرة المتسلسل الكمي في الوقت الحقيقي، واختبار ويسترن بلوت، واختبار Comet. أظهرت نتائجنا انخفاضًا كبيرًا في حجم الورم EST في الفئران المعالجة بجرعات منخفضة وعالية من مستخلص نبات القطف الخام مقارنة بحجم الورم EST في الفئران غير المعالجة. أظهرت تحليلات تفاعل البلمرة المتسلسل في الوقت الحقيقي وتحليل التعبير البروتيني أن مستخلص نبات القطف ينشط آليات موت الخلايا المبرمج، مما يؤدي إلى زيادة تنظيم تعبيرجينات p53 وCaspase 3 وcdc2 مع تقليل تنظيم تعبيرجينات *bcl2* على مستوى mRNA في أورام EST المعالجة في الفئران. وعلاوة على ذلك، أظهر اختبار Comet الإمكانات الجينية السامة لـ مستخلص نبات القطف على خلايا الورم الصلبة. تظهر نتائجنا أن نبات القطف قد يكون مصدرًا طبيعيًا واعدًا للعوامل المضادة لانتشار الأورام السرطانية . ومع ذلك، هناك حاجة إلى مزيد من التجارب السريرية لإثبات فعاليته وسلامته للاستخدام العلاجي. يوفر هذا البحث رؤى قيمة حول الآليات الجزيئية الكامنة وراء التأثيرات المضادة للأورام لمستخلص نبات القطف ويسلط الضوء على إمكاناته في استراتيجيات علاج السرطان.

الكلمات الدالة: Atriplex halimus، خلايا سرطان الاستسقاء في إيرليش، ورم إيرليش الصلب.