Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Purslane Seed Oil Extract and Doxorubicin: A Synergistic Approach against Liver Metastatic Ehrlich Ascites Carcinoma

Ibrahim S. Kamel*[a], Mona S. Gouida[b] , Mohammed S. El-Ghareeb[a], Hatem A. El-mezayen[c] , Lamia A. Ismail[a]

[a]*Department of Chemistry, Faculty of Science, Port Said University, Port Said, 8525502, Egypt*

[b]*University Children Hospital, Faculty of Medicine, Mansoura University, Mansoura, 7650001, Egypt*

[c]*Department of Chemistry, Faculty of Science, Helwan University, Helwan, 4020210, Egypt*

Abstract

Cancer is a global health challenge, with increasing cases and related deaths. Ehrlich Ascites carcinoma (EAC) is a well-established cancer model. Purslane (Portulaca oleracea L.) is a studied plant rich in bioactive compounds, including antioxidants, making it relevant in cancer research. Doxorubicin (DOX), a common anticancer drug, has potent effects but severe side effects. This study investigated the anti-tumor potential of Purslane seed oil methanolic extract (PSO) and its synergistic effects with the common anticancer drug doxorubicin (DOX) in EAC-bearing mice (n=64). Animals were randomized into eight groups (8/group): control, PSO alone, DOX alone, PSO+DOX, EAC control, EAC+PSO, EAC+DOX, and EAC+PSO+DOX. Following intraperitoneal injection of EAC cells ($2x10^6$), mice received 3 weeks of treatment with PSO (200 mg/kg/day) and DOX (4 mg/kg/week). PSO and DOX treatment alone demonstrated anti-tumor activity, with their combination exerting a synergistic effect. The PSO and DOX combination showed stronger anti-tumor effects, restoring antioxidant levels, reducing reactive oxygen species, and decreasing inflammation. PSO induced EAC cell apoptosis, increasing apoptotic marker caspase-3 and decreasing anti-apoptotic gene Bcl-2 expression. PSO and DOX significantly reduced EAC proliferation. In conclusion, PSO suppresses EAC tumor growth by improving oxidative balance, reducing inflammation, inducing apoptosis, and inhibiting proliferation. Further research is needed to clarify mechanisms and confirm its effectiveness against solid cancers as a dietary or medicinal agent.

Keywords: Portulaca oleracea L; Extract; Anti-tumor; Apoptosis; Ehrlich Ascites carcinoma.

1. Introduction

Ehrlich ascites cancer (EAC) stands as one of the most common experimental tumor models. It is an undifferentiated cancer induced in mice by injecting Ehrlich cells derived from a spontaneous mouse mammary tumor. These cells exhibit high transplantability, rapid growth, a short lifespan, and 100% tumor malignancy without tumor-specific transplantation antigen [1]. EAC shares similarities with human tumors, including sensitivity to chemotherapy, undifferentiation, and accelerated growth [2]. Widely employed as an experimental model, EAC aids in the study of various aspects of cancer biology, encompassing tumor growth, metastasis, angiogenesis, and drug resistance [3]. Around the world, breast tumor is one of the main and most reported and known cancers among females, and it is also the primary and most significant cause of female tumor-related deaths. For the reasons for modeling, experimental cancers have extraordinary significance and Ehrlich Ascites carcinoma (EAC) is one of the most common [4].

Currently, owing to their fewer complications and side effects, dietary natural compounds including herbs are gaining popularity in medicine development [5]. Portulaca oleracea L. (Purslane) is present all across the world and grows mainly in the subtropics and tropics, with its origins in South America and Africa [6]. Based on several reports, purslane is popular for its phytoremediation, nutritional, and medicinal effects [7]. Phytochemical studies indicated that purslane is one of the richest universal sources of ascorbic acid tocopherols, ω-3, ω-6 fatty

*Corresponding author e-mail: ibrahim.samy@sci.psu.edu.eg

Receive Date: 05 November 2023, Revise Date: 30 December 2023, Accept Date: 03 January 2024

DOI: 10.21608/EJCHEM.2024.246597.8816

©2024 National Information and Documentation Center (NIDOC)

acids, glutathione (GSH) and β-carotene indicating its antioxidant, and nutraceutical potential [6]. Moreover, alkaloids, flavonoids, anthocyanins, catecholamines, phenolic acids, lignans, terpenoids, and betalains are only a few of the specialized purslane metabolites [7]. For humans, these metabolites have been suggested offer potential health-enhancing advantages including their use in folk medicine and treatment of a variety of diseases, including cancers, diarrhea, urinary infections, headaches, kidney and cardiovascular diseases, ulcers, and diabetes [6,7]. Several studies, either on cultured cells or on animal models, suggest that the main constituents of purslane have great therapeutic features such as anxiolytic, antidepressant, neuroprotective, antimicrobial, antidiabetic, immunomodulatory, anti-inflammatory, antioxidant [7], and anticancer (including cytotoxicity and apoptogenic properties) [8].

Anticarcinogenic activities have been reported for Purslane. Investigations were carried out to screen the activities for antihepatocellular carcinoma, colon cancer, glioblastoma multiforme, ovarian cancer sarcoma, lung cancer, anti-cervical, gastric cancer, and pancreatic cancer. Purslane contains bioactive compounds with antioxidant properties, act on metastasis and invasion, modulate the immune system, and inhibit tumor formation [9]. Farshori et al. (2014) found the anti-cancer properties of purslane seed extract on human hepatocellular carcinoma (HepG2) cells. The results of this study revealed that the purslane seed extract remarkably mitigated the HepG2 cell viability in a dose-dependent manner. Furthermore, the purslane seed extract diminished the typical morphology and adhesion capacity of HepG2 cells. It was concluded that the purslane seed extract possesses anti-cancer properties in HepG2 cells [10].

It has been shown that purslane seed polysaccharides notably scavenge superoxide anion, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), and hydroxyl radicals. Moreover, the purslane seed polysaccharides prevented red blood cell (RBC) hemolysis as well as enhanced the spleen, thymocyte, T and B lymphocyte proliferation isolated from rats with ovarian cancer [11]. Chen et al. (2010) evaluated the cytotoxic effects of a sulfated derivative of purslane seed polysaccharide (POP) on liver (HepG2) and cervical (HeLa) cancer cell lines. The results showed that the POP sulfated derivative inhibited the growth of HeLa and HepG2 cells. It was concluded that sulfating POP increased its toxicity for tumor cells [12].

In cancer chemotherapy, antibiotics based on anthracycline, including doxorubicin (DOX), have shown widespread uses. DOX is the most frequently used anthracycline to treat many human solid cancers such as malignant lymphoma and acute leukemia [13,14]. DOX can suppress the cell's replication process by intercalating into DNA base pairs and crossing the tumor cellular membrane [13]. In contrast, clinical severe side effects are of major concern, including myelosuppression and cardiotoxicity and the liposomal drug delivery system can overcome this issue [13,14].

In this study, we attempted to explore the potential apoptogenic, anti-proliferative, and antioxidant effects of PSO methanolic extract on EAC mice model alone and in combination with DOX.

2. Material and methods

2.1 Animals

The experimental procedures were carried out following the guidelines set by the Mansoura University Animal Care and Use Committee (MU-ACUC), Mansoura University, Egypt, under approval code number: MU-ACUC (SC. PhD.22.09.1). A total of 64 healthy adult female Swiss albino mice, with a mean weight of 25±1.5 gm , were acquired from the animal house of the Egyptian Vaccine Institute in Giza, Cairo. The mice were housed under ambient room conditions with a temperature range of 23.0 ± 1.0°C, a relative humidity range of 40-80%, and a 12-hour light/12-hour dark cycle. They were supplied with a standard commercial diet containing all necessary nutrients and tap water ad libitum for two weeks before the start of the experiments.

2.2 Plant material and Extract Preparation

As a common vegetable, purslane was purchased from Al-Naqiti Herbs Company in Mansoura, Egypt. Voucher samples were kept in NAWAH Scientific Labs, Al-Mokattam, Cairo, Egypt. Under direct sunlight, the plant seeds were dried and cleaned by hand to remove any other contaminated small stalks, stones, and seeds. Then, the purslane seeds were dried in an oven for 12 hours at 50°C and powdered by a mechanical grinder. The powder with a particle size of 0.5-1 mm was kept in a vacuum dryer at NAWAH Scientific Labs, Al-Mokattam, Cairo, Egypt until use. The ground samples (3125 g) were mixed with 6 L methanol and then homogenized for 15 minutes using UltraTurrax T50 IKA Labotechnik.

The previous procedure was repeated for a second time. Then the extract was filtered and collected and dried under vacuum at 40°C producing a brown residue weighing 138.125 g. The extraction process was performed according to Abdullah et al. (2020) with some modifications [15].

2.3 Doxorubicin

Doxorubicin hydrochloride, synthesized at a concentration of 2 mg/ml by Pfizer Pharmaceutical Company, was purchased from El Tarshouby Pharmacy, Mansoura, Egypt.

2.4 Experimental design

EAC cells were acquired from the National Cancer Institute in Cairo, Egypt. These cells were consistently propagated in mice through intraperitoneal (ip) transplantation of 2 x 10^6 cells per mouse in a volume of 0.2 ml every 7 days. The treatment started after 2 weeks of transplantation [16]. Mice were divided into eight equal groups (8 animals/each): Group (A): Controls (normal diet). Group (B): PSO control (mice supplemented with a normal diet and a daily dose of PSO 200 mg/kg) [17]. Group (C): DOX control (mice supplemented with a normal diet and DOX (4 mg/Kg/week i.p.) [18]). Group (D): PSO + DOX control (mice supplemented with a normal diet, PSO, and DOX). Group (E): EAC tumor-bearing mice (supplemented with a normal diet). Group (F): EAC + PSO (after ip injection of EAC, tumor-bearing mice supplemented with a normal diet and a daily dose of PSO (200 mg/kg) for three weeks). Group (G): EAC + DOX (after ip injection of EAC, tumor-bearing mice supplemented with a normal diet and DOX (4 mg/Kg/week i.p.) for three weeks). Group (H): EAC + PSO + DOX (after ip injection of EAC, tumor-bearing mice supplemented with a normal diet, a daily dose of PSO (200 mg/kg), and DOX (4 mg/Kg/week i.p.) for three weeks).

2.5 Biochemical analysis

The liver was removed from the euthanized mice and rinsed with ice-cold phosphate-buffered saline (PBS). The liver was weighed and cut into small pieces (~1 mm) with a scalpel on ice. The liver pieces were transferred to a glass homogenizer tube and 10 volumes of Tris-EDTA buffer were added. The liver tissue was homogenized by grinding it with a glass pestle for 10 minutes on ice. The homogenate was transferred to a microcentrifuge tube and centrifuged at 12,000 x g for 15 minutes at 4°C. The supernatant was collected and stored at -80°C until further analysis.[19]. Superoxide dismutase (SOD) catalase and malondialdehyde (MDA) were performed on the liver tissue homogenate to measure the oxidative stress and antioxidant status of the liver. They were measured using Bio-diagnostic assay kits (Egypt) according to the instructions of manufacturers. SOD activity was measured by the xanthine oxidase method, which is based on the inhibition of the reduction of cytochrome c by superoxide generated from xanthine and xanthine oxidase [20]. Catalase activity was measured by the colorimetric method, which is based on the decrease in absorbance at 240 nm due to the consumption of hydrogen peroxide by catalase [21]. MDA content was measured by the thiobarbituric acid reactive substances (TBARS) method, which is based on the formation of a pink chromogen with thiobarbituric acid at high temperature and acidic pH [22]. In addition, blood samples were collected from the mice and centrifuged to obtain the serum and then the following assays were performed on the serum to measure the inflammatory and apoptotic markers of the mice. Serum reactive oxygen species (ROS) were quantified using dichlorofluorescein-diacetate (DCFH) (Sigma Aldrich, USA). ROS level was quantified by the dichlorofluorescein (DCF) method, which is based on the oxidation of non-fluorescent dichlorofluorescein-diacetate (DCFH-DA) to fluorescent dichlorofluorescein (DCF) by ROS in the presence of cellular esterases [23]. Serum TNF-α (RayBiotech, Parkway, LaneSuiteNorcross, USA), C-Reactive protein (CRP) (BD Biosciences, San Jose, CA) and Caspase-3 (RayBiotech, Parkway, LaneSuiteNorcross, USA) were assessed using commercial ELISA kits, in accordance with the guidelines and instructions provided by the manufacturer.

2.6 Flow cytometry analysis for Ki-67 and Bcl-2

100 µl of liver tissue sample was combined with 1 ml of cold PBS in a centrifuge tube and left for 10 minutes at 37°C for fixation. The mixture was then centrifuged for 5 minutes, and the supernatant was discarded. Cells were stained in two tubes, one with 5 µl of anti-Ki-67 antibody, and another one with 5 µl of anti-Bcl-2 antibody. Both samples were kept in a dark environment and allowed to incubate for fifteen minutes at room temperature (25°C). After incubation, the cells were rinsed with PBS (containing 1% BSA) and then preserved by fixation with paraformaldehyde (4% in PBS). The intensity of

cleaved Ki-67 and Bcl-2 was measured using flow cytometry. The stained cells were acquired on a flow cytometer (Accuri C6 by Becton Dickinson) and analyzed using Accuri C6 software [24].

2.7 Flow cytometry analysis of apoptosis by Annexin V-FITC kit:

100 µl of cell suspension from liver tissue sample was washed with 1 ml of PBS in a centrifuge tube and spun for 5 minutes at 1800 rpm. Then the supernatant was discarded and the pellet of cells was stained with 5 µl of Annexin V-FITC and 5 µl of propidium iodide (PI) in 100 µl of 1X Binding Buffer. The samples were kept in a dark place and allowed to incubate for fifteen minutes at room temperature. After incubation, the cells were ready for acquisition by flow cytometry Accuri C6 Becton Dickinson. The percentage of cells analysed by Accuri C6 software represented the quadrant dot plot to perform the four phases of viable, early, late and finally necrosis in each group [24].

2.8 Relative gene expression

Liver tissues were harvested from the euthanized mice and rinsed with ice-cold PBS. The tissues were weighed and cut into small pieces (~1 mm) with a scalpel on ice. The tissue pieces were transferred to a glass homogenizer tube and 10 volumes of RLT buffer (provided in the RNeasy Mini Kit) were added. The tissues were homogenized by grinding them with a glass pestle for 10 minutes on ice. The homogenate was transferred to a microcentrifuge tube and centrifuged at 12,000 x g for 15 minutes at 4°C. The supernatant was collected and stored at -80°C until further analysis. Total RNA content was extracted from cells via the RNeasy Mini Kit (Qiagen/BioRad). Using 10 ng total RNAs/each sample, expressions of Bcl-2 and Ki-67 genes were quantified through cDNA synthesis by reverse transcription using Reverse Transcriptase kit (Biosystems, USA). Subsequently in a 48-well plate, the produced cDNA was amplified (Syber Green I PCR Master Kit) using the Step One instrument (Biosystems, USA), as a following: 95ºC (10 minutes for enzyme activation), 40 cycles (15 seconds at 95ºC, 20 seconds at 55ºC and 30 seconds at 72ºC for amplification). By the ΔCt method, gene expression changes were normalized relative to GAPDH (housekeeping gene) mean critical threshold (CT) values. Primers specific for each gene was added and mRNA levels were quantified using the 2 ΔΔCq method. Experiments were performed in triplicate.

2.9 Histopathological analysis

Histopathological examinations were performed to analyze the cellular structures of EAC control and treated groups. Samples were collected from the intraperitoneal mice and washed with PBS. They were then smeared onto slides and stained with Hematoxylin & Eosin (H&E) using standard procedures. The stained slides were observed under a light microscope at 40x and 100x magnifications. Typical tumor cells, normal cells with minimal EAC damage, eosinophilic swallowing bodies, condensed and fragmented nuclei, as well as numerous pyknotic cells, were identified. The images were captured using a digital camera attached to the microscope and labelled [25].

2.10 Statistical analysis

Variables were represented by mean ± SD. In all analyses, SPSS 20 and GraphPad Prism 8 At each comparison, statistical significance difference was determined by one-way ANOVA followed by Tukey-Kramer as post-Hoc test and was represented by *P* value. $P<0.05$ was considered significant.

3. Results

3.1 Anti-inflammatory and anti-oxidant activity of PSO

Ip injection of EAC into mice caused a significant ($P<0.0001$) increase in inflammatory markers including CRP (60.8±3.0 vs. 15.2±0.2 ng/mL) and TNF-α (33.6±1.8 vs. 3.3±0.15 pg/mL) compared to normal control mice (Table 1). In hepatic tissues, it is also caused significant increase in lipid peroxidation (MDA; 9.9±0.31 vs. 7.4±0.14 nmol/g. tissue) and conversely significant decrease in antioxidants catalase (73.3±2.5 vs. 130±0.5 U/g. tissue) and SOD (31.6±1.1 vs. 57.8±0.4 U/g. tissue) (Table 1). Total ROS production was increased significantly owing to ip injection EAC (51±0.44 vs. 5±0.21 pg/mL). Mice' oral administration of PSO extract alone or in combination with DOX revealed a significant ($P<0.0001$) decrease in CRP, TNF-α, ROS and MDA as well as increase in antioxidants compared to animals received ip injection of EAC (Table 1).

3.2 Effect on apoptosis

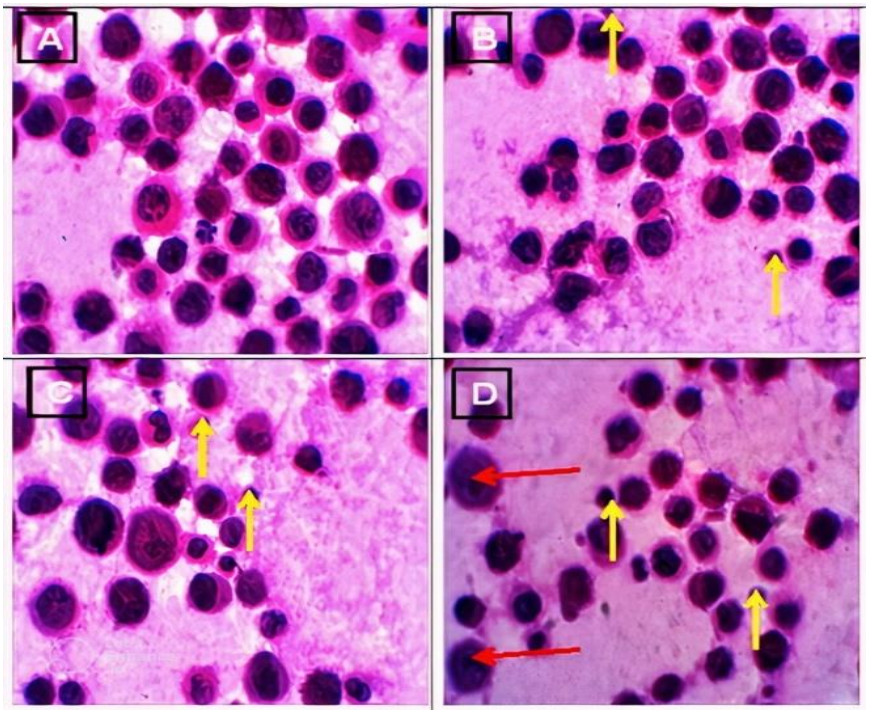
As indicated by microscoic magnification, EAC tumor cells were characterized with moderate sized nuclei and abundant dark stained and basophilic cytoplasm (Fig. 1A).

Table 1 Ameliorative effects on antioxidants, lipid peroxidation and inflammation markers

Variable	Controls	PSO	DOX	PSO+DOX	EAC	EAC+PSO	EAC+DOX	EAC+DOX+PSO	*P* value
CRP (ng/mL)	15.2±0.2	20±2.8	27.8±1.3	24.5±1.9	60.8±3.0*	26.5±2.5@	47.8±2.1	31.5±1.7@	0.0001
TNF-α (pg/mL)	3.3±0.15	4.5±0.3	4.9±0.99	3.0±0.75	33.6±1.8*	27.3±2.2@	21±1.7	15.3±0.94@	0.0001
MDA (nmol/g. tissue)	7.4±0.14	7.4±0.1	7.5±0.1	7.5±0.7	9.9±0.31*	8.7±0.23@	9.1±0.1	8.9±0.14@	0.0001
ROS (pg/mL)	5±0.21	10.3±0.5	12.3±1.0	9±0.82	51±0.44*	41.8±2.2@	34.8±3.7	24.3±0.5@	0.0001
Catalase (U/g. tissue)	130±0.5	133±0.2	134±1.5	133.1±5	73.3±2.5*	107.2±0.92@	114.9±0.8	108.8±1.3@	0.0001
SOD (U/g. tissue)	57.8±0.4	55.2±0.2	56.1±0.4	53.6±2.5	31.6±1.1*	44.3±0.6@	45.3±0.4	45.9±0.3@	0.0001

Values represent mean ± SD. Differences were assessed using ANOVA test. *Significantly different from control group at p< 0.05. @ Significantly different from control group at p< 0.05. CRP: C-reactive protein; TNF: Tumor necrosis factor; MDA: Malondialdehyde; ROS: Reactive oxygen species; SOD: Superoxide dismutase. EAC: Ehrlich Ascites Carcinoma; PSO: Purslane seed oil extract; DOX: Doxorubicin.

Figure 1. Photomicrographs of H&E staining of EAC control and different experimental groups (A) EAC control group showing typical tumor cells with round shape and definite nucleus. (B and C) EAC treated with PSO extract and EAC treated with DOX showing nearly normal cells with few damage EAC (yellow arrow). (D) EAC treated with DOX+PSO extract showing numerous eosinophilic swallowing bodies (Red arrow) with condensed and fragmented nuclei and numerous pyknotic cells (yellow arrow).



Treatment with PSO (Fig. 1B), DOX (Fig. 1C) and their combination (Fig. 1D) decreased size and number of EAC tumor cells and appeared with azurophilic lytic bodies and with a

light stained eosinophilic cytoplasm. Also, these treatments exhibited apoptotic features including shrinkage, fragmenting nuclei, nuclear chromatin compaction, apoptotic bodies, and blebbing plasma membrane.

To further confirm that PSO provoked cell apoptosis on EAC cells, apoptotic cells were then quantified by Annexin V flow cytometric analysis (Fig. 2). The percentage of early apoptotic cells represented by the upper right (UR) quadrant was significantly ($P<0.0001$) increased by treatment with PSO, DOX and their combination (Table 2). Also, detection of an apoptotic marker caspase-3 revealed that ip injection of EAC significantly decreased caspase-3 and PSO and DOX treatments reverse this EAC anti-apoptotic feature (Table 2).

Figure 2. Flow cytometry analysis of annexin-V for the control and EAC, PSO extract and DOX treated groups. EAC: Ehrlich Ascites Carcinoma; PSO: Purslane seed oil extract; DOX: Doxorubicin. The upper left (UL) quadrant indicates necrotic cells, the left lower (LL) quadrant corresponds to healthy cells, the upper right (UR) quadrant represents early apoptotic cells, and the lower right (LR) quadrant represents late apoptotic cells.

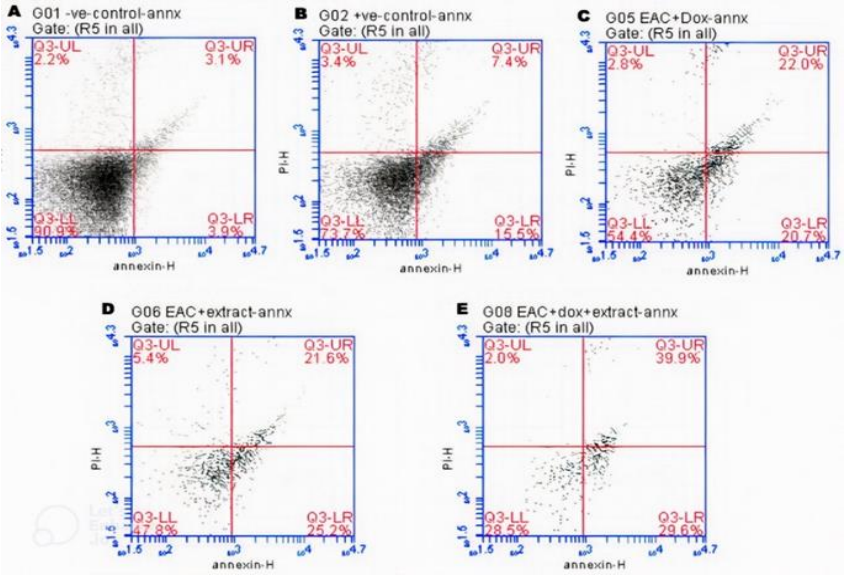


Table 2 Effect on apoptosis and cell proliferation markers

Variable		Controls	PSO	DOX	PSO+DOX	EAC	EAC+PSO	EAC+DOX	EAC+DOX+PSO	*P* value
Annexin-V	LL	91.1±0.2	57.1±0.3	60.3±0.22	44.9±7.8	75.3±1.9	54±5.3	51.2±3.8	29.8±2.0	0.0001
	LR	3.8±0.1	20.7±0.1	24.7±0.1	29.6±0.3	14.2±1.7	23.3±1.8	20.8±0.1	30±1.0	0.0001
	UL	2.2±0.03	7±0.1	3.03±0.1	3.3±0.4	3.5±0.2	4.9±0.4	2.8±0.1	2.1±0.2	0.0001
	UR	3.0±0.15	15.2±0.4	11.9±0.4	22.2±4.3	7±0.6	17.9±3.1	25.3±3.8	38.2±2.9	0.0001
Caspase-3 (ng/mL)		20.3±0.5	18±0.13	17±0.5	14.3±1	11.3±2.5	29.3±0.96	29.8±2.4	40.3±1.3	0.0001
Ki-67*		17.4±0.9	18.7±0.6	20±3.9	21.8±17.5	84.5±1.5	42.3±5.3	41±18.7	30.8±5.9	0.0001
BCL-2*		28.4±0.2	26.3±0.8	29.7±0.4	19.7±7	65.5±3.8	42.2±1.4	37.6±6	38.2±1.2	0.0001

Values represent mean±SD. $P<0.05$ is significant. Differences were assessed using ANOVA test. The upper left (UL) quadrant indicates necrotic cells, the left lower (LL) quadrant corresponds to healthy cells, the upper right (UR) quadrant represents early apoptotic cells, and the lower right (LR) quadrant represents late apoptotic cells. EAC: Ehrlich Ascites Carcinoma; PSO: Purslane seed oil extract; DOX: Doxorubicin. * Results obtained from labelling using flow cytometric analysis.

3.3 Effect on EAC proliferative capability

Also, proliferative capability of EAC was significantly decreased using PSO extract and DOX as reported using Ki67 nuclear proliferation marker flow cytometric analysis (Table 2) and also down regulation of relative gene expression (Fig. 3A). Moreover, ip injection of EAC caused significant increase in anti-apoptotic gene BCL-2 as reported by flow cytometric analysis (Table 2) and relative gene expression (Fig. 3B).

4. Discussion

Beside its nutritional benefits and potential uses in folk remedy, purslane has anticancer effects owing to its antioxidant contents, protective effect against DNA damage and tumor cells proliferation and enhance tumor cells apoptosis [11]. There are very limited data about anticancer effects of PSO extract particullary against EAC-induced mammry gland carcinoma; thus, this study aimed to explore the potential apoptogenic, anti-proliferative and antioxidant effects of PSO methanolic extract on EAC mice model alone and in combination with common comercial anticancer drug DOX.

In this study, mice subjected to ip injection of EAC showed a significant ($P<0.0001$) increase in inflammatory markers including CRP and TNF-α. In addition, EAC implantation resulted in a notable elevation in hepatic lipid peroxidation (MDA) and conversely significant decrease in hepatic antioxidants catalase and SOD and increased the total ROS production. Oral injection of PSO extract alone or in combination with DOX revealed a significant ($P<0.0001$) decrease in CRP, TNF-α, ROS and MDA as well as increase in antioxidants compared to animals received ip injection of EAC.

In addition to serving as a source of primary metabolites, purslane also contains varying quantities of specific secondary metabolites, including organic acids, phenolic acids, terpenoids, cardiac glycosides, flavonoids, tannins, saponins, alkaloids, ascorbic acid, tocopherols, ω-3 and ω-6 fatty acids, glutathione (GSH), and β-carotene. These various bioactive compounds, present in varying concentrations, are likely to be primarily responsible for its antioxidant and anti-inflammatory properties [6,11,26].

Our results correlate with many studies that have reported that purslane attenuates the oxidative state by increasing antioxidant levels and decreasing lipid peroxidation [27]. Also, PSO anti-inflammatory activity reported in our study is in agreement with many other studies. In vascular diseases, Lee et al. reported that purslane significantly repressed TNF-α, intracellular ROS production and the vascular inflammation process [28]. Also, Xu et al. and Kim et al. reported that purslane significantly diminished the secretion of inflammatory factors including cyclooxygenase 2, prostaglandin E2, IL-6, TNF-α and NO synthase [29,30]. As a tool to reduce CRP levels, a recent meta-analysis concluded a positive effect of purslane administration [31].

In this study, we examined cytotoxicity and apoptogenic properties of PSO extract on EAC tumor cells. Treatment with PSO and DOX exhibited apoptotic features including decreased size and number of EAC cells, presence of azurophilic lytic bodies, light stained eosinophilic cytoplasm, shrinkage, fragmenting nuclei, apoptotic bodies and blebbing plasma membrane. This apoptogenic effect confirmed with Annexin V flow cytometric analysis that revealed that the percentage of early apoptotic cells was significantly ($P<0.0001$) increased by treatment with PSO, DOX and their combination. Also, PSO reverse the EAC anti-apoptotic related decrease of caspase-3 and reverse the increase in anti-apoptotic gene Bcl-2 as reported by flow cytometric analysis and relative gene expression.

In line with our results, Rahimi et al reported the effect of hydro-rehethanolic extract of purslane on glioblastoma cells (U-87) and they found the extract had great cytotoxicity effect and enhance apoptosis induction of tumor cells [32]. In cervical carcinoma, polysaccharides derived from purslane induce apoptosis of HeLa and U14 cells through increasing Bax (apoptotic protein) and decreasing Bcl-2 [33]. Also, Zhao et al findings showed that oral polysaccharide of purslane induced apoptosis in U14 tumor bearing mice through increase mitochondrial Ca2+ and Bcl-2 expression, reduce caspase 3 expression and prevented cytochrome c mitochondrial release [34]. In a concentration-dependent manner, Chen et al suggested that sulfated derivatives of purslane polysaccharide inhibits cell proliferation and progression in rats with ovarian cancer [12]. Moreover in human hepatocellular carcinoma (HCC) (HEPG-2) cells, nano forms of purslane extracts blocked cells proliferation and induced apoptosis [14]. Al-Sheddi et al. reported cytotoxic effects of PSO on HepG2 and lung cancer A-549 cell lines [35].

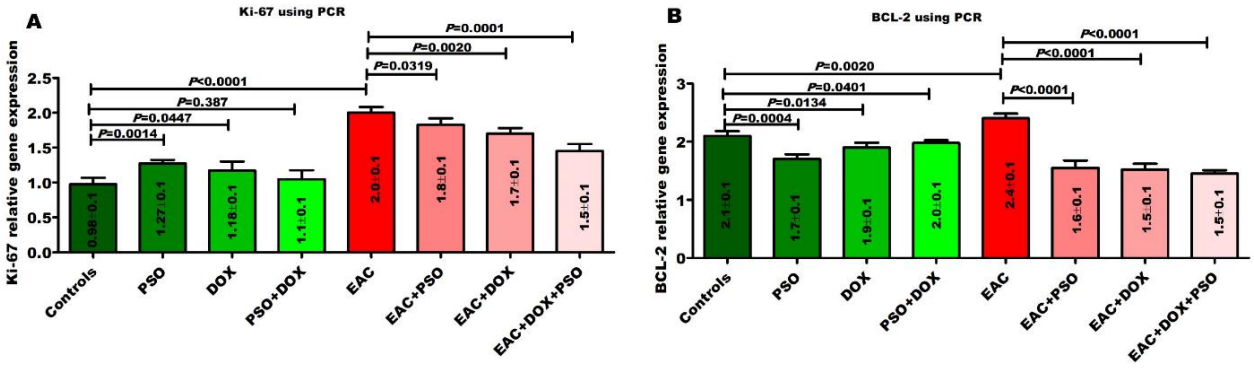


Figure 3. Effect of EAC, PSO extract and DOX supplementation on (A) Ki-67 relative and (B) Bcl-2 gene expression. Differences between groups were assessed using ANOVA test followed by Tukey test as a post-hoc test. $P<0.05$ is significant. EAC: Ehrlich Ascites Carcinoma; PSO: Purslane seed oil extract; DOX: Doxorubicin.

Portulacerebroside A (PCA), cerebroside compound isolated from purslane, was reported as a promising candidate for treating HCC as it significantly diminished HCC HCCLM3 cell viability in concentration- and time-dependent manner [36]. PCA increased caspase-3 and caspase-9, stimulates the release of mitochondrial cytochrome C, disrupted mitochondrial membrane permeability and elevates apoptotic cells rates [36]. Also, apigenin, another purslane constituent, was reported to significantly and potently inhibits glioblastoma cell proliferation by inhibition of AKT/mTOR signaling pathways and inducing the apoptosis [37]. In addition, anti-glioma activity of kaempferol was suggested through induction of apoptosis [38]. Therefore, polyphenolic compounds such as polysaccharides, and apigenin and kaempferol may accompany PSO apoptogenic and cytotoxic effects on EAC.

Ki-67 antibodies is closely related to tumor malignancy degree and it is considered as well tumour proliferation marker [39]. In this study, proliferative capability of EAC was significantly decreased using PSO extract and DOX as reported using Ki-67 flow cytometric analysis and also down regulation of relative gene expression. This finding is in agreement with results of Jia et al as they found that purslane polysaccharides boost the immune effectiveness of dendritic cell vaccines. and decreased ki-67 level of breast cancer cells [40].

3. Conclusion

PSO methanolic extract could supress the EAC tumor growth by intraperitoneally administration and the mechanism may be related to ameliorating the oxidative state and antioxidant system, reducing inflammation and inflammatory markers, inducing tumor cell apoptosis and constraining tumor cell proliferation. These results enhanced understanding that PSO may be used as a drug or dietary agent for strengthening the human body and immunity against different common solid cancers but further studies are urgently needed to support the idea and deeply focusing on related mechanisms.

4. Declaration

The authors declare the work is not published anywhere else.

5. Conflict of interests

There is no conflict of interest.

6. Funding

No funding for the research work was carried out.

7. Authors' contributions

Each of the authors has made a significant contribution to the paper.

8. Acknowledgments

We sincerely thank Prof. Dr. Hanaa Ali Hassan, Professor of Animal Physiology & Ecology at Mansoura University, for her invaluable contributions, and valuable suggestions to our research.

9. References

[1] Latifi A, Luwor RB, Bilandzic M, Nazaretian S, Stenvers K, Pyman J, Zhu H, Thompson EW, Quinn MA, Findlay JK. Isolation and characterization of Tumor cells from the Ascites of Ovarian cancer patients: molecular phenotype of chemoresistant ovarian tumors. PLoS One. 2012;7:10. https://doi.org/10.1371/journal.pone.0046858

[2] El-Missiry M, Abd El-Aziz A. Influence of melatonin on proliferation and antioxidant system

in Ehrlich ascites carcinoma cells. Cancer Lett. 2000;2:119–25. https://doi.org/10.1016/S0304-3835(99)00366-3

[3] Aldayel, T.S., Gad El Hak, H.N., Nafie, M.S. et al. Evaluation of antioxidant, anti-inflammatory, anticancer activities and molecular docking of Moringa oleifera seed oil extract against experimental model of Ehrlich ascites carcinoma in Swiss female albino mice. BMC Complement Med Ther 2023;23:457. https://doi.org/10.1186/s12906-023-04279-z

[4] Alotaibi B, Tousson E, El-Masry TA, Altwaijry N, Saleh A. Ehrlich ascites carcinoma as model for studying the cardiac protective effects of curcumin nanoparticles against cardiac damage in female mice. Environ Toxicol 2021;36:105–13. https://doi.org/10.1002/tox.23016.

[5] Ghasemzadeh Rahbardar M, Hosseinzadeh H. Effects of rosmarinic acid on nervous system disorders: an updated review. Naunyn Schmiedebergs Arch Pharmacol 2020;393:1779–95. https://doi.org/10.1007/s00210-020-01935-w.

[6] Kumar A, Sreedharan S, Kashyap AK, Singh P, Ramchiary N. A review on bioactive phytochemicals and ethnopharmacological potential of purslane (Portulaca oleracea L.). Heliyon 2022;8. https://doi.org/10.1016/j.heliyon.2021.e08669.

[7] Jalali J, Rahbardar MG. Ameliorative effects of Portulaca oleracea L. (purslane) and its active constituents on nervous system disorders: A review. Iran J Basic Med Sci 2023;26:2–12. https://doi.org/10.22038/IJBMS.2022.65764.14464.

[8] Rahimi VB, Rakhshandeh H, Raucci F, Buono B, Shirazinia R, Kermani AS, et al. Anti-Inflammatory and Anti-Oxidant Activity of Portulaca oleracea Extract on LPS-Induced Rat Lung Injury. Molecules 2019;24:139. https://doi.org/10.3390/molecules24010139.

[9] de Souza, P. G., Rosenthal, A., Ayres, E. M. M., & Teodoro, A. J. Potential functional food products and molecular mechanisms of Portulaca oleracea L. on anticancer activity: A review. Oxidative medicine and cellular longevity, 2022.

[10] Farshori NN, Al-Sheddi ES, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, Siddiqui MA. Cytotoxicity Assessments of Portulaca oleracea and Petroselinum sativum Seed Extracts on Human Hepatocellular Carcinoma Cells (HepG2) Asian Pac J Cancer Prev. 2014;15(16):6633–8. 10.7314/APJCP.2014.15.16.6633.

[11] YouGuo, Chen, Shen ZongJi, and Chen XiaoPing. "Evaluation of free radicals scavenging and immunity-modulatory activities of Purslane polysaccharides." International journal of biological macromolecules 2009;45(5):448–52

[12] Chen T, Wang J, Li Y, Shen J, Zhao T, Zhang H. Sulfated modification and cytotoxicity of Portulaca oleracea L. polysaccharides. Glycoconj J. 2010;27(6):635–42. 10.1007/s10719-010-9307-0.

[13] Elbialy NS, Mady MM. Ehrlich tumor inhibition using doxorubicin containing liposomes. Saudi Pharmaceutical Journal 2015;23:182–7. https://doi.org/10.1016/j.jsps.2014.07.003.

[14] Utkusavas A, Gurel Gurevin E, Yilmazer N, Uvez A, Oztay F, Bulut H, et al. Effects of combined administration of doxorubicin and chloroquine on lung pathology in mice with solid Ehrlich ascites carcinoma. Biotechnic and Histochemistry 2022;97:555–66. https://doi.org/10.1080/10520295.2022.2036369.

[15] Abdullah, M. S., Kusumaningtyas, R. D. The extraction of purslane (Portulaca oleracea L.) using alcohol solvents 48% and its utilization as a source of encapsulated omega-3 oil. AIP Conference Proceedings, 2020;2197(1), 080004

[16] El-Missiry MA, Othman AI, Amer MA, Mohamed E. Ottelione A inhibited proliferation of Ehrlich ascites carcinoma cells in mice. Chem Biol Interact 2012;200:119–27. https://doi.org/10.1016/j.cbi.2012.10.013.

[17] Farag, O.M., Abd-Elsalam, R.M., Ogaly, H.A. et al. Metabolomic Profiling and Neuroprotective Effects of Purslane Seeds Extract Against Acrylamide Toxicity in Rat's Brain. Neurochem Res 46, 819–842 (2021). https://doi.org/10.1007/s11064-020-03209-6

[18] Hullin, R., Métrich, M., Sarre, A., Basquin, D., Maillard, M., Regamey, J., & Martin, D. "Diverging effects of enalapril or eplerenone in primary prevention against doxorubicin-induced cardiotoxicity." Cardiovascular Research 114.2 (2018): 272-281.

[19] Zhang D. Methods and techniques for tissue homogenization: A comprehensive review. J Cell Sci Mut. 2023;7(4):152

[20] Nishikimi M, Appaji Rao N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun

1972;46:849–54. https://doi.org/10.1016/S0006-291X(72)80218-3.

[21] Aebi. Catalase in Vivo. Methods Enzymol 1984;105:121–6.

[22] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8. https://doi.org/10.1016/0003-2697(79)90738-3.

[23] Korystov, Y.N., Shaposhnikova, V.V., Korystova, A.F. and Emel'yanov, M.O. " Detection of reactive oxygen species induced by radiation in cells using the dichlorofluorescein assay" Radiation Research, 2007;168 (2), pp. 226–232. https://doi.org/10.1667/RR0925.1

[24] Tribukait, B., Moberger, G. and Zetterberg, A. (1975) Methodological Aspects for Rapid Flow Cytofluorometry for DNA Analysis of Human Urinary Bladder Cells. In: Haenen, C., Hillen, H. and Wessels, S., Eds., Pulse Cytophotometry Part I, European Press Medicon, Ghent, 55-60.

[25] Bancroft JD, Cook HC (1994) Manual of Histological Techniques and Their Diagnostic Application. Edinburgh, London, New York, Tokyo: Churchill Livingstone, pp. 23–26.

[26] Al-Quwaie DA, Allohibi A, Aljadani M, Alghamdi AM, Alharbi AA, Baty RS, et al. Characterization of Portulaca oleracea Whole Plant: Evaluating Antioxidant, Anticancer, Antibacterial, and Antiviral Activities and Application as Quality Enhancer in Yogurt. Molecules 2023;28. https://doi.org/10.3390/molecules28155859.

[27] Wang CQ, Yang GQ. Betacyanins from Portulaca oleracea L. ameliorate cognition deficits and attenuate oxidative damage induced by D-galactose in the brains of senescent mice. Phytomedicine 2010;17:527–32. https://doi.org/10.1016/j.phymed.2009.09.006.

[28] Lee AS, Kim JS, Lee YJ, Kang DG, Lee HS. Anti-TNF-α activity of Portulaca oleracea in vascular endothelial cells. Int J Mol Sci 2012;13:5628–44. https://doi.org/10.3390/ijms13055628.

[29] Xu L, Ying Z, Wei W, Hao D, Wang H, Zhang W, et al. A novel alkaloid from Portulaca oleracea L. Nat Prod Res 2017;31:902–8. https://doi.org/10.1080/14786419.2016.1253081.

[30] Kim C, Park P, Choe S, Kimz T, … JL-KJO, 2009 undefined. Anti-oxidative and Anti-inflammatory Effects of Protulaca O/eracea on the LPS-stimulated AGS Cells. HantopicCom

[31] Zhu Y, Huang Y, Santos HO, de Oliveira CVC, Zhou H, Tang N. Effects of purslane supplementation on C-reactive protein levels and biomarkers of oxidative stress as marks for metabolic syndrome: A systematic review and meta-analysis of randomized controlled trials. Phytotherapy Research 2021;35:5477–86. https://doi.org/10.1002/ptr.7182.

[32] Rahimi VB, Mousavi SH, Haghighi S, Soheili-Far S, Askari VR. Cytotoxicity and apoptogenic properties of the standardized extract of portulaca oleracea on glioblastoma multiforme cancer cell line (U-87): A mechanistic study. EXCLI J 2019;18:165–86. https://doi.org/10.17179/excli2019-1063.

[33] Zhao R, Gao X, Cai Y, Shao X, Jia G, Huang Y, et al. Antitumor activity of Portulaca oleracea L. polysaccharides against cervical carcinoma in vitro and in vivo. Carbohydr Polym 2013;96:376–83. https://doi.org/10.1016/j.carbpol.2013.04.023.

[34] Zhao R, Shao X, Jia G, Huang Y, Liu Z, Song B, et al. Anti-cervical carcinoma effect of Portulaca oleracea L. polysaccharides by oral administration on intestinal dendritic cells. BMC Complement Altern Med 2019;19:1–10. https://doi.org/10.1186/s12906-019-2582-9.

[35] Al-Sheddi ES, Farshori NN, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, Siddiqui MA. Portulaca oleracea seed oil exerts cytotoxic effects on human liver cancer (HepG2) and human lung cancer (A-549) cell lines. Asian Pacific Journal of Cancer Prevention 2015;16:3383–7. https://doi.org/10.7314/APJCP.2015.16.8.3383.

[36] Zheng GY, Qu LP, Yue XQ, Gu W, Zhang H, Xin HL. Portulacerebroside A induces apoptosis via activation of the mitochondrial death pathway in human liver cancer HCCLM3 cells. Phytochem Lett 2014;7:77–84. https://doi.org/10.1016/j.phytol.2013.10.005.

[37] Stump TA, Santee BN, Williams LP, Kunze RA, Heinze CE, Huseman ED, et al. The antiproliferative and apoptotic effects of apigenin on glioblastoma cells. Journal of Pharmacy and Pharmacology 2017;69:907–16. https://doi.org/10.1111/jphp.12718.

[38] Sharma V, Joseph C, Ghosh S, Agarwal A, Mishra MK, Sen E. Kaempferol induces apoptosis

in glioblastoma cells through oxidative stress. Mol Cancer Ther 2007;6:2544–53. https://doi.org/10.1158/1535-7163.MCT-06-0788.

[39] Menon SS, Guruvayoorappan C, Sakthivel KM, Rasmi RR. Ki-67 protein as a tumour proliferation marker. Clinica Chimica Acta 2019;491:39–45. https://doi.org/10.1016/j.cca.2019.01.011.

[40] Jia G, Shao X, Zhao R, Zhang T, Zhou X, Yang Y, et al. Portulaca oleracea L. polysaccharides enhance the immune efficacy of dendritic cell vaccine for breast cancer. Food Funct 2021;12:4046–59. https://doi.org/10.1039/d0fo02522d.