Alfarama Journal of Basic & Applied Sciences

Faculty of Science Port Said University

https://ajbas.journals.ekb.eg
ajbas@sci.psu.edu.eg
http://sci.psu.edu.eg/en/

July 2024, Volume 5, Issue III

DOI:https://doi.org/10.21608/AJBAS.2024.261360.1207

ISSN 2682-275X

Submitted: 11/01/2024
Accepted: 13/02/2024

Pages: 341 - 349

Purslane Seed Oil: A Promising Adjuvant for Doxorubicin in Ehrlich Ascites Carcinoma Therapy

Ibrahim S. Kamel[1,*], Mohammed S. El-Ghareeb[1], Mona S. Gouida[2] Hatem A. El-mezayen[3] , Lamia A. Ismail[1]

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

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

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

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

ABSTRACT

Purslane seed oil (PSO) is a herbal extract with antioxidant and anti-inflammatory properties that may enhance the efficacy and reduce the toxicity of doxorubicin (DOX), a potent anticancer drug. This study investigated the effects of PSO and DOX on the cell cycle and kidney and liver function in Ehrlich ascites carcinoma (EAC) mice, a widely used experimental model of cancer. Forty female mice were randomly assigned to five equal groups (n = 8/group) with distinct treatment regimens: control, EAC control, EAC+PSO, EAC+DOX, and EAC+PSO+DOX. After EAC cell injection ($2x10^6$), mice received 3 weeks of treatment with PSO (200 mg/kg/day) and DOX (4 mg/kg/week). The cell cycle analysis and biochemical assays performed on EAC-bearing mice showed that PSO and DOX exerted different and synergistic effects on the cell cycle and apoptosis of EAC cells, and that their combination reduced the deterioration of the kidney and liver function parameters in EAC-bearing mice. These findings suggest that PSO could be a potential complementary agent to DOX in the treatment of EAC and other cancers, as it could enhance the antitumor and organ-protective effects of DOX.

Key Words:

Portulaca oleracea L.; Extract; Ehrlich Ascites carcinoma; Cell cycle; Doxorubicin

1. INTRODUCTION

Cancer is characterized by the accelerated and unregulated development of anomalous cells, which can coalesce to form a tumor or disseminate throughout the body, indicating irregular growth at diverse locations [1]. Current scientific investigations are primarily directed towards identifying both natural and synthetic compounds for potential use in preventing and/or treating cancer. Within modern medicine, chemotherapy is recognized as the most efficacious approach to cancer treatment [2].

Regrettably, existing cancer chemotherapeutic agents exert subtle adverse effects on host cells, particularly impacting bone marrow, epithelial tissues, reticulo-endothelial system, and gonads [3]. Numerous antineoplastic agents induce severe and prolonged toxicities, potentially irreversible, particularly affecting the heart, lungs, and kidneys [4]. To minimize unwanted toxicity, a strategy involves the utilization of novel natural products that may operate through distinct mechanisms and/or induce fewer or different side effects. Consequently, natural products are now regarded as exceptionally valuable in the development of potent anticancer drugs with minimal host cell toxicity and notable antioxidant potential [5].

The Ehrlich tumor, initially characterized as a spontaneous murine mammary adenocarcinoma, presents as a rapidly growing carcinoma with highly aggressive behavior, capable of thriving in nearly all mouse strains [4]. In its ascitic form, it serves as a transplantable tumor model for investigating the antitumor effects of various substances [6].

The use of natural compounds or herbal extracts with anticancer and antioxidant properties represents a promising strategy in cancer research. These agents offer multiple benefits, including enhancing the immune system, modulating the tumor microenvironment, inhibiting angiogenesis and metastasis, and sensitizing cancer cells to chemotherapy [7]. Portulaca oleracea L. (Purslane), a medicinal plant with potential anticancer effects, is highlighted for its diverse pharmacological activities [8]. Purslane seed oil (PSO) methanolic extract, rich in flavonoids, is particularly noteworthy [9]. Flavonoids, such as kaempferol, apigenin, myricetin, quercetin, and luteolin, present in PSO, have been shown to exert anticancer effects in various cancer models. The flavonoids modulate cellular signaling pathways, impacting gene expression related to cell cycle regulation, apoptosis, angiogenesis, metastasis, and inflammation [10]. Additionally, they enhance cancer cell sensitivity to chemotherapy and protect normal tissues from oxidative damage [10]. The purslane powder extract contains nineteen identified phenolic acids, with ellagic acid being the dominant compound [11]. Overall, pursuing these natural compounds for anticancer interventions show promise in both in vivo and in vitro settings [12]. This study explored the antineoplastic potential of Purslane seed oil (PSO), focusing on a methanolic extract, in a Swiss albino mice model of Ehrlich ascites carcinoma.

2. METHODS AND MATERIALS

2.1 Animals

This study adhered to the rigorous ethical guidelines established by the Mansoura University Animal Care and Use Committee (MU-ACUC) under approval number MU-ACUC (SC. PhD.22.09.1). Forty healthy, adult female Swiss albino mice with an average weight of 25 ± 1.5 grams were obtained from the Egyptian Vaccine Institute's animal house in Giza, Cairo. Prior to the experiment, the mice underwent a two-week acclimatization period under controlled conditions. Specifically, the environment maintained 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. During this period, the mice had unlimited access to a standard commercial diet purchased from El-Nasr Company for Chemicals and Fertilizers, Egypt. The diet composition is as follows: crude protein 18%, crude fiber 4%, crude fat 3%, ash 8%, and moisture 10% and received unrestricted tap water.

2.2 Plant material and Extract Preparation

Fresh *Portulaca oleracea* (purslane) was obtained from Al-Naqiti Herbs Company, Mansoura, Egypt. Voucher specimens were deposited at NAWAH Scientific Labs, Al-Mokattam, Cairo, Egypt. Seeds were air-dried, manually cleaned of debris, and further dried in an oven at 50°C for 12 hours. Following mechanical grinding, the resulting powder (particle size 0.5-1 mm) was vacuum-preserved at NAWAH Labs. Ground samples (3.125 kg) were subjected to sequential methanol extractions (6 L each) using an UltraTurrax T50 homogenizer for 15 minutes. The combined filtrates were vacuum-dried at 40°C, yielding a 138.125 g brown residue. This modified protocol was based on Abdullah et al. (2020) [13].

2.3 Doxorubicin

Doxorubicin hydrochloride, a commercially available chemotherapeutic agent, was employed in this study. The drug was sourced from El Tarshouby Pharmacy, located in Mansoura, Egypt. The specific product utilized was a pre-synthesized solution prepared by Pfizer Pharmaceutical Company at a concentration of 2 mg/ml.

2.4 Experimental design

The present study employed the Ehrlich ascites carcinoma (EAC) cell line procured from the National Cancer Institute, Cairo, Egypt. This model was established through regular intraperitoneal (i.p.) transplantation of 2 x 10^6 cells per mouse (0.2 ml volume) every 7 days, ensuring consistent tumor development [14]. Following two weeks of tumor establishment, forty female mice were randomly assigned to five equal groups (n = 8/group) with distinct treatment regimens: Group A: Control: Mice received a standard diet only. Group B: EAC Tumor: Mice received a standard diet and developed EAC tumors following injection. Group C: EAC + PSO: EAC tumor-bearing mice received a standard diet and additional PSO (200 mg/kg/day) **by oral gavage** for three weeks [15]. Group D: EAC + DOX: EAC tumor-bearing mice received a standard diet and DOX (4 mg/kg/week i.p.) for three weeks [16]. Group E: EAC + PSO + DOX: EAC tumor-bearing mice received a standard diet, PSO, and DOX for three weeks.

2.5 Animal handling and samples collection

With sodium pentobarbital (3%), mice were completely anesthetized and the chest was opened. By cardiac puncture, blood samples were collected and then, to gets sera, were separated by centrifugation (15 minutes; 3500 rpm). Serum samples were stored at -20˚C for the biochemical analyses. The liver was excised and promptly blotted with filter paper to eliminate any remaining blood residues.

2.6 Biochemical analyses

Employing the Reitman and Frankel method, Serum levels of aspartate (AST) and alanine (ALT) aminotransferases were obtained [17]. Commercially available Bio-diagnostic kits (Egypt) were utilized to quantify serum albumin, alkaline phosphatase (ALP) [18], creatinine [19] and urea [20] were evaluated according to the manufacturer's instructions. Quantitative enzyme-linked fluorescent immunometric assays on an automated mini-VIDAS® platform (BioMérieux, France) were used to measure serum α-Fetoprotein (AFP) levels according to the approach proposed by Aldubayan et al. [21]. Serum sodium and potassium concentration was assayed by a colorimetric method using a commercial kit supplied by Diatek Company [22].

2.7 Flow cytometric analysis of cell cycle

Liver tissues were homogenized in PBS to prepare cell suspensions, which were then cleared of debris and clumps. The prepared cell suspension underwent centrifugation for 5 min at 300 rpm, and the supernatant was decanted. The pellet was then resuspended in 5 ml of PBS and centrifuged for an additional 5 min at 300 rpm. Subsequently, the pellet was suspended in 1 ml of propidium iodide (PI) solution and incubated in the dark at room temperature for 30 min. The fluorescence of the cells was measured in the flow cytometer, with PI excitation bound to DNA at a maximum of 536 nm and emission at 617 nm [23].

2.8 Statistical analysis

For all statistical analyses, SPSS 25 and GraphPad Prism 9 were used. Normal distributed data were represented as mean±SD. Statistical significance difference was determined by one-way ANOVA followed by TukeyKramer as post-Hoc test. $P<0.05$ was considered significant [24], [25].

3. RESULTS

3.1 Effects on Liver and Kidney Functions in EAC-Implanted Mice:

Compared to the normal control group, EAC ip implantation induced liver damage, leading to a significant ($P<0.0001$) increase in liver enzyme activities (ALT, AST, ALP) and AFP levels, along with a decrease in serum albumin (Table 1). Mice receiving PSO extract alone or in combination with DOX exhibited a significant ($P<0.05$) decrease in liver enzymes and AFP compared to EAC-implanted animals. The combined PSO+DOX treatment showed a more pronounced effect than individual supplementation of PSO and DOX.

Regarding kidney functions and electrolytes, EAC implantation resulted in a significant ($P<0.0001$) increase in serum creatinine, urea, and potassium levels, as well as a decrease in serum sodium levels. Administration of DOX and PSO methanolic extract significantly ($P<0.05$) reduced the worsening of impaired kidney functions and blood electrolytes caused by EAC alone, demonstrating their potential protective effects (Table 1).

3.2 Cell Cycle Analysis

The cell cycle analysis revealed that PSO and DOX had significant effects ($P<0.0001$) on the cell cycle distribution and apoptosis of EAC cells. As shown in Figures (1,2) and, Table 2, the EAC group had a low SubG1% with Mean± SD (23.62±1.2), indicating a low level of apoptosis. However, treatment with PSO or DOX alone increased the SubG1% to Mean± SD (60.05±1.2) and with Mean± SD (58.25±2) respectively, indicating a high level of apoptosis and reduced cell viability. The combination of PSO and DOX further increased the SubG1% to with Mean± SD (70.3±1.59), indicating a synergistic effect of both agents on inducing apoptosis and decreasing cell survival. PSO and DOX, alone or combined, decreased G0/G1, S, and G2/M cell populations, implying reduced proliferation and increased death. This suggests both agents modulate EAC cell cycle and apoptosis, with their combination exhibiting potent antitumor activity.

4. DISCUSSION

The present study aimed to evaluate the effects of PSO methanolic extract and DOX on EAC cell cycle, kidney and liver functions in mice. EAC is a widely used experimental model of cancer that mimics the characteristics of human tumors, such as rapid growth, high invasiveness, and resistance to chemotherapy [26]. EAC cells affect hepatocytes through the ascetic fluid accumulation and by hepatic enzymes leakage such as ALT, AST and ALP into serum [27], [28]. Previous studies have reported that EAC tumors can lead to kidney damage by increasing creatinine and urea levels, contributing to elevated tubular atrophy and glomerular capillary permeability, which plays a key role in the development of renal failure [29], [30]. DOX is a potent anticancer drug that inhibits DNA synthesis and induces apoptosis in cancer cells, but also causes severe side effects, such as cardiotoxicity, nephrotoxicity, and hepatotoxicity [4]. Therefore, there is a need to find alternative or complementary agents that can enhance the efficacy of DOX and reduce its toxicity.

PSO is a rich source of omega-3 fatty acids, antioxidants, and phytochemicals that have been reported to possess various pharmacological properties, such as anti-inflammatory, antidiabetic, antihyperlipidemic, and anticancer activities [31]. In this study, we found that PSO alone or in combination with DOX significantly reduced the tumor burden in EAC-bearing mice, indicating its antitumor potential. Moreover, PSO and PSO+DOX treatment prevented the decline of liver and kidney function parameters compared to the EAC group, suggesting its protective role against EAC-induced organ damage. These findings are consistent with previous studies that showed the beneficial effects of PSO on liver and kidney functions in diabetic and hyperlipidemic rats [31], [32].

The mechanism of action of PSO and DOX on EAC cells was further investigated by analyzing the cell cycle distribution and apoptosis. We observed that PSO treatment induced a significant increase in the subG1 phase, which represents the apoptotic cells, and a decrease in the S phase, which represents the DNA synthesis phase, in EAC cells. These results indicate that PSO induced cell cycle arrest and apoptosis in EAC cells, possibly by modulating the expression of cell cycle regulators and apoptotic proteins. Previous studies have shown that PSO can induce apoptosis in various cancer cell lines, such as

breast, colon, and prostate cancer, by activating the intrinsic and extrinsic pathways of apoptosis, involving the mitochondrial membrane potential, caspases, Bcl-2 family, and death receptors [33], [34], [35], [36].

On the other hand, DOX treatment induced a significant increase in the G0/G1 phase, which represents the resting phase, and a decrease in the G2/M phase, which represents the mitotic phase, in EAC cells. These results indicate that DOX induced cell cycle arrest at the G0/G1 phase and inhibited the mitotic entry of EAC cells, possibly by interfering with the DNA replication and repair processes. Previous studies have shown that DOX can induce G0/G1 arrest in various cancer cell lines, such as leukemia, lung, and ovarian cancer, by downregulating the expression of cyclin D1, cyclin E, CDK2, and CDK4, and upregulating the expression of p21 and p27 [3], [16], [37].

Interestingly, the combination of PSO and DOX showed a synergistic effect on EAC cells, as it induced a significant decrease in both the G0/G1 and S phases, and a significant increase in both the subG1 and G2/M phases, compared to the single treatments. These results indicate that the combination of PSO and DOX enhanced the cell cycle arrest and apoptosis in EAC cells, possibly by affecting multiple targets and pathways involved in the regulation of cell proliferation and survival.

5. CONCLUSION

This study showed that purslane seed oil (PSO) and doxorubicin (DOX) had different and synergistic effects on the cell cycle and apoptosis of Ehrlich ascites carcinoma (EAC) cells, and that they prevented the deterioration of the kidney and liver function in EAC mice. These findings suggested that PSO could be a potential adjuvant to DOX in cancer therapy, but further studies were needed to understand the molecular mechanisms and pathways involved.

Table 1. Ameliorative effects on liver and kidney functions and electrolytes

Variable	Controls	EAC	EAC+PSO	EAC+DOX	EAC+DOX+PSO
ALT (U/L)	48.6±0.4	152.7±1.8*	97.5±0.4**	98.6±1.3**	83.3±2.1**
AST (U/L)	39.8±0.3	117.7±5.5*	85.2±1.5**	88.9±2.1**	79.4±0.94**
ALP (U/L)	58.5±0.3	184.5±3.3*	87.8±1.3**	91.4±2.9**	73.7±0.5**
Albumin (g/dL)	4.2±0.1	3.0±0.2*	3.2±0.3*	3.2±0.05*	3.2±0.1*
AFP (ng/mL)	10.3±0.2	83.1±2.1*	41.7±1.3**	37.8±1.3**	30.2±2.1**
Creatinine (mg/dL)	0.93±0.2	1.3±.08*	1.1±0.06**	1.48±0.13**	1.8±0.08**
Urea (mg/dL)	26.4±0.3	31.6±1.2*	32.4±0.43*	35.7±1.45*	39±1.6*
Potassium (mEq/L)	3.0±0.2	5.8±0.5*	4.2±0.15**	3.8±0.3**	3.9±0.3**
Sodium (mEq/L)	131.2±1	114.8±1.3*	120.8±1.3**	123.6±1.3**	125.2±2.1**

Table 2. The distribution of cell cycle phases among different groups

Groups	SubG1%	G0/G1%	S%	G2/M%
EAC	**23.62±1.2**	**40.45±0.6**	**30.37±1.2**	**5.5±0.6**
EAC + PSO	**60.05±1.2***	**21.1±1.3***	**16.72±0.98***	**2.12±0.19****
EAC + Dox	**58.25±2***	**22.7±1.96***	**16.75±0.76***	**2.3±0.63****
EAC + Dox + PSO	**70.3±1.59***	**19.15±1.5***	**8.575±0.5***	**1.975±0.62****

Data are presented as mean ± SD.*P<0.0001, highly significant difference compared to the control group **P<0.05, significant difference compared to the EAC group. **EAC**: Ehrlich Ascites Carcinoma; **PSO**: Purslane seed oil extract; **DOX**: Doxorubicin.

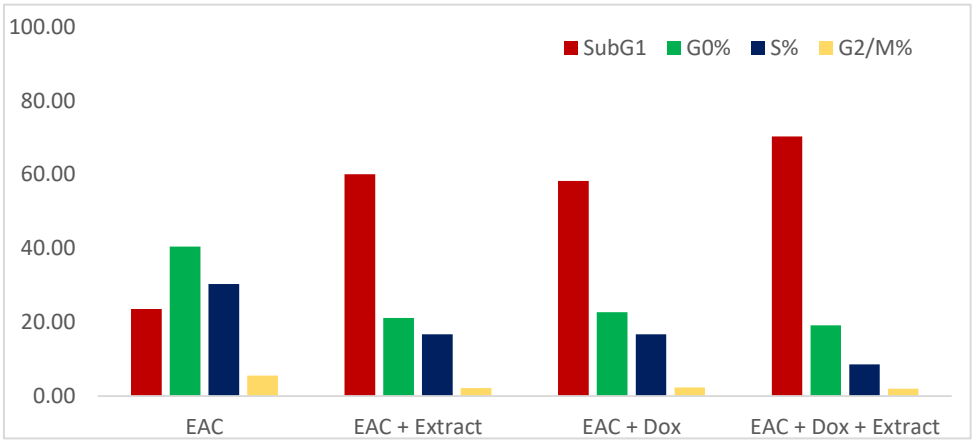

Figure 1. Histogram represent the correlation between cell cycle stages and the studied (EAC) groups.

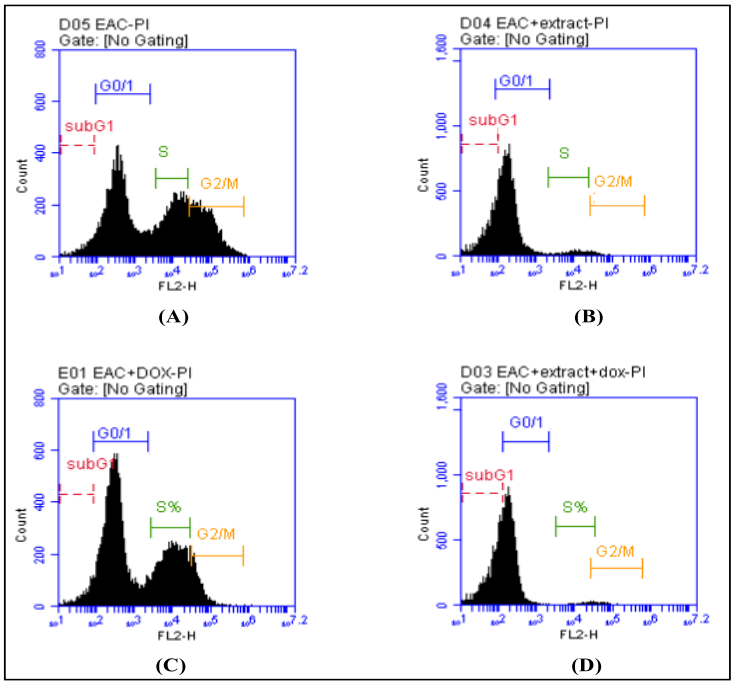

Figure 2. Computerized flow cytometric results represent the correlation between cell cycle stages and the studied (EAC) groups. **(A)**: **EAC**; **(B)**: **EAC + PSO**; **(C): EAC + DOX**; **(D): EAC + PSO + DOX**. **EAC**: Ehrlich Ascites Carcinoma; **PSO**: Purslane seed oil extract; **DOX**: Doxorubicin.

Conflict of interest: There is no conflict of interest.

6. REFERENCES

[1] Jassim, E. P. Rahrmann, B. D. Simons, and R. J. Gilbertson, "Cancers make their own luck: theories of cancer origins," *Nat Rev Cancer*, vol. 23, no. 10, pp. 710–724, Jul. 2023, doi: 10.1038/s41568-023-00602-5.

[2] D. Wodarz and N. Komarova, "Can loss of apoptosis protect against cancer?," *Trends in Genetics*, vol. 23, no. 5, pp. 232–237, 2007, doi: 10.1016/j.tig.2007.03.005.

[3] E. S. M. El-Sayed, A. M. Mansour, and M. S. Abdul-Hameed, "Thymol and Carvacrol Prevent Doxorubicin-Induced Cardiotoxicity by Abrogation of Oxidative Stress, Inflammation, and Apoptosis in Rats," *J Biochem Mol Toxicol*, vol. 30, no. 1, pp. 37–44, Jan. 2016, doi: 10.1002/jbt.21740.

[4] N. S. Elbialy and M. M. Mady, "Ehrlich tumor inhibition using doxorubicin containing liposomes," *Saudi Pharmaceutical Journal*, vol. 23, no. 2, pp. 182–187, 2015, doi: 10.1016/j.jsps.2014.07.003.

[5] D. J. Newman and G. M. Cragg, "Natural products as sources of new drugs over the last 25 years," *J Nat Prod*, vol. 70, no. 3, pp. 461–477, Mar. 2007, doi: 10.1021/np068054v.

[6] N. G. Sannappa Gowda, V. D. Shiragannavar, S. C. Prabhuswamimath, S. Tuladhar, S. B. Chidambaram, and P. K. Santhekadur, "Ehrlich Ascites carcinoma mice model for studying liver inflammation and fibrosis," *Advances in Cancer Biology - Metastasis*, vol. 4, no. 10002, p. 9, 2022, doi: 10.1016/j.adcanc.2022.100029.

[7] Y. X. Zhou, H. L. Xin, K. Rahman, S. J. Wang, C. Peng, and H. Zhang, "Portulaca oleracea L.: A review of phytochemistry and pharmacological effects," *Biomed Res Int*, vol. 2015, 2015, doi: 10.1155/2015/925631.

[8] J. W. Yoon, S. S. Ham, and H. S. Jun, "Portulaca oleracea and tumor cell growth," *Official Gazette of the United States Patent and Trademark Office Patents*, vol. 1219, no. 2, p. 1472, 1999, [Online]. Available: https://patents.google.com/patent/US5869060A/en

[9] E. S. Elkhayat, S. R. M. Ibrahim, and M. A. Aziz, "Portulene, a new diterpene from Portulaca oleracea L.," *J Asian Nat Prod Res*, vol. 10, no. 11, pp. 1039–1043, 2008, doi: 10.1080/10286020802320590.

[10] H. B. Nayaka, R. L. Londonkar, and M. K. Umesh, "Evaluation of potential antifertility activity oftotal flavonoids, isolated from Portulaca oleracea L on female albino rats," *Int J Pharmtech Res*, vol. 6, no. 2, pp. 783–793, 2014, [Online]. Available: https://www.academia.edu/download/35616263/Evaluation_of_Potential_Antifertility_activity_of.pdf

[11] R. N. Azizah, A. Emelda, I. Asmaliani, I. Ahmad, and M. Fawwaz, "Total Phenolic, Flavonoids, and Carotenoids Content and Anti-Obesity Activity of Purslane Herb (Portulaca oleracea L.) Ethanol Extract," *Pharmacognosy Journal*, vol. 14, no. 1, pp. 8–13, 2022, doi: 10.5530/pj.2022.14.2.

[12] M. Kalachaveedu, R. Senthil, S. Azhagiyamanavalan, R. Ravi, H. Meenakshisundaram, and A. Dharmarajan, *Traditional medicine herbs as natural product matrices in cancer chemoprevention: A trans pharmacological perspective (scoping review)*, vol. 37, no. 4. Phytotherapy Research, 2023. doi: 10.1002/ptr.7747.

[13] M. S. Abdullah and R. D. Kusumaningtyas, "The extraction of purslane (Portulaca oleracea L.) using alcohol solvents 48 % and its utilization as a source of encapsulated omega-3 oil," *AIP Conf Proc*, vol. 2197, 2020, doi: 10.1063/1.5140944.

[14] M. A. El-Missiry, A. I. Othman, M. A. Amer, and E. Mohamed, "Ottelione A inhibited proliferation of Ehrlich ascites carcinoma cells in mice," *Chem Biol Interact*, vol. 200, no. 2–3, pp. 119–127, Dec. 2012, doi: 10.1016/j.cbi.2012.10.013.

[15] O. M. Farag *et al.*, "Metabolomic Profiling and Neuroprotective Effects of Purslane Seeds Extract Against Acrylamide Toxicity in Rat's Brain," *Neurochem Res*, vol. 46, no. 4, pp. 819–842, Apr. 2021, doi: 10.1007/s11064-020-03209-6.

[16] R. Hullin *et al.*, "Diverging effects of enalapril or eplerenone in primary prevention against doxorubicin-induced cardiotoxicity," *Cardiovasc Res*, vol. 114, no. 2, pp. 272–281, 2018, doi: 10.1093/cvr/cvx162.

[17] S. REITMAN and S. FRANKEL, "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases.," *Am J Clin Pathol*, vol. 28, no. 1, pp. 56–63, 1957, doi: 10.1093/ajcp/28.1.56.

[18] A. Belfield and D. M. Goldberg, "Normal ranges and diagnostic value of serum 5′nucleotidase and alkaline phosphatase activities in infancy," *Arch Dis Child*, vol. 46, no. 250, pp. 842–846, 1971, doi: 10.1136/adc.46.250.842.

[19] J. SCHIRMEISTER, H. WILLMANN, and H. KIEFER, "Critical Evaluation of Plasma Creatinine As a Test of Glomerulus," *Verh Dtsch Ges Inn Med*, vol. 70, pp. 678–681, 1964, doi: 10.1007/978-3-642-96030-7_106.

[20] J. K. FAWCETT and J. E. SCOTT, "A rapid and precise method for the determination of urea.," *J Clin Pathol*, vol. 13, no. 2, pp. 156–159, 1960, doi: 10.1136/jcp.13.2.156.

[21] M. A. Aldubayan, R. M. Elgharabawy, A. S. Ahmed, and E. Tousson, "Antineoplastic activity and curative role of avenanthramides against the growth of Ehrlich solid tumors in mice," *Oxid Med Cell Longev*, vol. 2019, 2019, doi: 10.1155/2019/5162687.

[22] E. Tousson, A. El-Atrsh, M. Mansour, and A. Abdallah, "Modulatory effects of Saussurea lappa root aqueous extract against ethephon-induced kidney toxicity in male rats," *Environ Toxicol*, vol. 34, no. 12, pp. 1277–1284, Dec. 2019, doi: 10.1002/tox.22828.

[23] H. Shapiro, *Practical flow cytometry*. 2005. Accessed: Jan. 25, 2024. [Online]. Available: https://books.google.com/books?hl=en&lr=&id=JhSyimPKuJwC&oi=fnd&pg=PR7&dq=Shapiro,+H.+(1988).+%22Practical+flow+cytometry.+New+York:+Alan+R.+Liss.,+Inc.+353+p.%22&ots=OTyYzQUNPD&sig=YPOmLVG1C-MD5_VdFWXqaKq8icg

[24] W. T. Morgan, "A review of eight statistics software packages for general use," *American Statistician*, vol. 52, no. 1, p. 70, 1998, doi: 10.1080/00031305.1998.10480542.

[25] G. H. Freeman, K. A. Gomez, and A. A. Gomez, *Statistical Procedures for Agricultural Research.*, vol. 41, no. 1. 1985. doi: 10.2307/2530673.

[26] E. Tousson, E. Hafez, M. M. Abo Gazia, S. B. Salem, and T. F. Mutar, "Hepatic ameliorative role of vitamin B17 against Ehrlich ascites carcinoma–induced liver toxicity," *Environmental Science and Pollution Research*, vol. 27, no. 9, pp. 9236–9246, 2020, doi: 10.1007/s11356-019-06528-6.

[27] D. S. Morsi *et al.*, "Anti-proliferative and immunomodulatory potencies of cinnamon oil on Ehrlich ascites carcinoma bearing mice," *Sci Rep*, vol. 12, no. 1, Dec. 2022, doi: 10.1038/s41598-022-14770-1.

[28] P. P. Patil *et al.*, "Effect of Theobroma cacao L. on the Efficacy and Toxicity of Doxorubicin in Mice Bearing Ehrlich Ascites Carcinoma," *Antioxidants*, vol. 11, no. 6, p. 6, 2022, doi: 10.3390/antiox11061094.

[29] T. F. Mutar, E. Tousson, E. Hafez, M. Abo Gazia, and S. B. Salem, "Ameliorative effects of vitamin B17 on the kidney against Ehrlich ascites carcinoma induced renal toxicity in mice," *Environ Toxicol*, vol. 35, no. 4, pp. 528–537, 2020, doi: 10.1002/tox.22888.

[30] N. Saleh *et al.*, "Protective and Therapeutic Efficacy of Hesperidin versus Cisplatin against Ehrlich Ascites Carcinoma-Induced Renal Damage in Mice," *Pharmaceuticals*, vol. 15, no. 3, 2022, doi: 10.3390/ph15030294.

[31] L. A. A. Barakat and R. H. Mahmoud, "The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats," *N Am J Med Sci*, vol. 3, no. 9, pp. 351–357, 2011, doi: 10.4297/najms.2011.3351.

[32] M. Askaripour, S. R. F. Tabatabaei, F. Hosseini, and H. N. Varzi, "Effect of purslane (Portulaca oleracea) on renal ischemia/reperfusion injury in rat," *Int J Pharm Pharm Sci*, vol. 7, no. 1, pp. 467–471, 2015.

[33] M. A. Dkhil, A. E. A. Moniem, S. Al-Quraishy, and R. A. Saleh, "Antioxidant effect of purslane (Portulaca oleracea) and its mechanism of action," *Journal of Medicinal Plants Research*, vol. 5, no. 9, pp. 1589–1593, 2011, [Online]. Available: https://academicjournals.org/journal/JMPR/article-full-text-pdf/C1D5B4017817

[34] C. YouGuo, S. ZongJi, and C. XiaoPing, "Evaluation of free radicals scavenging and immunity-modulatory activities of Purslane polysaccharides," *Int J Biol Macromol*, vol. 45, no. 5, pp. 448–452, 2009, doi: 10.1016/j.ijbiomac.2009.07.009.

[35] I. Bay, K. Fisheries, and I. Bay, "Effects of Aqueous Purslane (Portulaca Oleracea) Extract and Fish Oil on Gentamicin Nephrotoxicity in Albino Rats," *Nat Sci (East Lansing)*, vol. 9, no. 2, pp. 47–62,

2011, [Online]. Available: https://www.researchgate.net/profile/Walaa-Hozayen/publication/303910661_Effects_of_Aqueous_Purslane_Portulaca_Oleracea_Extract_and_Fish_Oil_on_Gentamicin_Nephrotoxicity_in_Albino_Rats/links/57658d0508ae1658e2f4a6e3/Effects-of-Aqueous-Purslane-Portulaca-O

[36] S. Petropoulos, A. Karkanis, N. Martins, and I. C. F. R. Ferreira, "Phytochemical composition and bioactive compounds of common purslane (Portulaca oleracea L.) as affected by crop management practices," *Trends Food Sci Technol*, vol. 55, pp. 1–10, 2016, doi: 10.1016/j.tifs.2016.06.010.

[37] S. M. Attia, S. A. Bakheet, and N. M. Al-Rasheed, "Proanthocyanidins produce significant attenuation of doxorubicin-induced mutagenicity via suppression of oxidative stress," *Oxid Med Cell Longev*, vol. 3, no. 6, 2010, doi: 10.4161/oxim.3.6.14418.