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Anti-tumor effects and cellular mechanisms of *Pistacia atlantica* methanolic extract against Ehrlich solid tumor in mice

Aishah E. Albalawi[1], Norah A. Althobaiti[2], Reem Hasaballah Alhasani[3], Sultan F. Alnomasy[4]✉

[1]*Faculty of Science, Department of Biology, University of Tabuk, Tabuk 47913, Saudi Arabia*

[2]*Biology Department, College of Science and Humanities, Al Quwaiiyah, Shaqra University, Al Quwaiiyah 19257, Saudi Arabia*

[3]*Department of Biology, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia*

[4]*Department of Medical Laboratories Sciences, College of Applied Medical Sciences, Al-Quwayiyah, Shaqra University, Riyadh, Saudi Arabia*

ABSTRACT

Objective: To assess the anti-tumor effects of *Pistacia atlantica* methanolic extract (PAME) compared with cyclophosphamide against Ehrlich solid tumors in mice.

Methods: Swiss albino mice (n=40) were divided into five groups: normal control mice, mice with Ehrlich solid tumors treated with normal saline, mice with Ehrlich solid tumors treated with cyclophosphamide intraperitoneally once a day for 14 d, or 50 mg/kg or 100 mg/kg PAME orally once a day for 14 d. Tumor growth inhibition, body weight, tumor markers, liver and kidney enzymes, oxidative stress markers, antioxidant enzymes, tumor necrosis factor-alpha level (TNF-α), and apoptosis-regulatory gene expression were evaluated.

Results: Treatment of mice bearing Ehrlich solid tumors with PAME at 50 and 100 mg/kg orally significantly decreased tumor volume, body weight, tumor markers, liver and kidney enzymes, oxidative stress markers and TNF-α level in comparison with mice with Ehrlich solid tumors receiving normal saline. whereas PAME at 50 and 100 mg/kg/day significantly elevated the level of antioxidant enzymes (P<0.05).

Conclusions: *Pistacia atlantica* methanolic extract has potent antitumor activity in mice. Therefore, the extract might be considered as an alternative anticancer agent against tumors, however, additional studies especially in the clinical setting are required to confirm this finding.

KEYWORDS: Cancer; Ehrlich solid tumors; Treatment; *Pistacia atlantica*; Herbal medicines; Natural product; *in vivo*; Mice

1. Introduction

Among the many diseases that threaten human health and life, cancer is one of the most important reasons for death in the world[1]. The disease is characterized by the disruption of the normal cell cycle, which causes unplanned cell growth and subsequently the absence of differentiation, characterized as malignant developments[2]. Chemotherapy has long been one of the most important cancer therapies, which can be used alone or in combination with other treatments, such as surgical processes and radiotherapies[3]. At present, a wide range of chemical drugs are used to prevent and treat various types of cancers; however, the studies demonstrated that the current synthetic medications are accompanied by some adverse side effects such as damage to the normal tissues and cells, inhibition of bone marrow function,

Significance

At present, a wide range of chemical drugs are used to prevent and treat various types of cancers; however, the studies demonstrated that the current synthetic drugs are associated with some adverse side effects. The results of the present study revealed that *Pistacia atlantica* methanolic extract might be considered as an alternative anticancer agent against tumors, and additional studies especially in the clinical setting are required to confirm this finding.

✉To whom correspondence may be addressed. E-mail: s.alnomasy@su.edu.sa

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nausea, vomiting, alopecia, *etc*[4,5]. Therefore, the search for a new anticancer drug, especially from high-performance and low-toxicity sources, has always been of interest to researchers. In recent years, herbs and their derivatives and products have attracted attention due to their high effectiveness, low toxicity, availability, and cheapness, as alternative drugs in the treatment of cancers[6,7].

Wild pistachio [*Pistacia atlantica* (*P. atlantica*) Desf.], from the Anacardiaceae family, broadly grows in central Asia, particularly in Saudi Arabia, Syria, *etc*[8]. The plant has various therapeutic potential as traditional medicine and is used to treat or improve some diseases and conditions such as digestive problems, vomiting, diuretic, demulcent, dyspepsia, skin diseases, wound healing, disorders of liver and kidney, gastrointestinal disorders, toothache diseases, *etc*[9]. In addition, it also has several other pharmacological properties such as antinociceptive, antidiabetic, anti-inflammatory, antiproliferative, hepatoprotective, antioxidant, immunomodulatory, antimicrobial, *etc*[9,10]. Several studies also have reported the *in vitro* and *in vivo* anticancer activity of different parts of this plant against colonic adenocarcinoma (COLO205), human gastric carcinoma (AGS), human colorectal adenocarcinoma cell (HT29 cells), cervix adenocarcinoma (HeLa cells), *etc*[10-12]. The Ehrlich tumor as a murine mammary adenocarcinoma is a solid form of the tumor which is broadly used in the assessment of efficacy and innovation of new anti-tumor agents[13,14]. The current survey was designed to assess the anti-tumor effects of *P. atlantica* methanolic extract (PAME) compared with cyclophosphamide (CP) against the Ehrlich solid tumors (EST) in mice.

2. Materials and methods

2.1. Reagents and drugs

All chemicals, Folin-Ciocalteau's reagent, as well as Mayer and Dragendorff's were obtained from Sigma-Aldrich (St. Louis, MO). MyBio-Source Mice Carcinoembryonic Antigen Elisa Kit was obtained from MyBio-Source, San Diego, USA. Liver and kidney enzymes kits were prepared from Roche, Germany. The mini-VIDAS® AFP kit was prepared from bioMérieux, Marcyl'Etoile, France. The oxidative stress kits, the antioxidant enzymes kits, and TNF-α ELISA kit were purchased from Abcam, USA. RNeasy tissue kit and the complementary DNA (cDNA) synthesis kit were prepared from Qiagen Co, Germany. All the chemicals used were of analytical grade.

2.2. Plant material

Fruits of *P. atlantica* were purchased from a marketplace in Riyadh city, Saudi Arabia and after being determined by a botanist, a sample of voucher was deposited at the herbarium of Shaqra University, Saudi Arabia for further experiments.

2.3. Preparation of methanolic extract

By percolation method, 200 g of the dried fruits were extracted with methanol for 72 h at 21 ℃. Next, the obtained extracts were passed over a filter paper and were evaporated by a rotary evaporator in a vacuum at 55 ℃ and preserved at −20 ℃ until examination[15].

2.4. Phytochemical analysis

The primary phytochemical analysis of the PAME was done to test alkaloids, tannins, glycosides, saponins, and flavonoids, including Mayer and Dragendorff's reagents to identify the alkaloids, Mg and HCl for identification of flavonoids, 1% gelatin along with 10% NaCl solutions to determine the tannin, the combination of $FeCl_2$ and H_2SO_4 to examine the glycosides, and saponin determined by the capacity to produce suds[16].

2.4.1. Total phenol content

Total phenol content was studied using the Folin-Ciocalteau's reagent colorimetric method using gallic acid as a standard[17]. In brief, 0.2 mL of PAME was added to Folin-Ciocalteu solution and then 2 mL of sodium carbonate was added to the combination. Followed by the incubation (30 min) in the darkness, the absorbance of the mixture was recorded at 760 nm using a spectrophotometer (Jenway 6715, Cole-Parmer, Eaton Socon, UK); the results were described as mg gallic acid (GAE)/g dry weight.

2.4.2. Total flavonoid content

The content of total flavonoid was assessed based on the technique explained by Phuyal *et al.*[18] using aluminum chloride ($AlCl_3$ 2%) colorimetric and quercetin as a standard. Briefly, 0.2 mL of extract or standard solution was added to 0.2 mL of aluminum chloride and 0.1 mL of 33% aqueous acetic acid and stirred well. In the end, the combination was reached a volume of 5 mL with ethanol. Lastly, the absorbance was measured at 430 nm and the total flavonoid was gained through a standard curve as mg quercetin equivalent per gram dry weight (mg QE/g DW).

2.4.3. Tannin condensed contents

The method described by Broadhurst and Jones[19] was used to determine the tannin condensed contents of PAME. After mixing the extract and control with vanillin-HCl (5 mL), the absorbance was measured at 510 nm. The content was presented as mg catechin (CE)/g DW.

2.5. Anti-tumor effects of PAME

2.5.1. Animals

Fifty-six female Swiss albino mice weighing 20-25 g and aged 6–8 weeks old were used to establish the animal model of EST. Mice were held in a colony room under a 12:12 h light/ dark cycle at (21 ± 2) ℃ with *ad libitum* access to food and water.

2.5.2. The Ehrlich ascites tumor cell line and induction of EST in mice

The cell line of the Ehrlich ascites tumor was prepared from the American Type Tissue Culture Collection (Manassas, USA) and

was adjusted into 2.5×10^6 cells/mL in sterile saline solution using a Neubauer hemocytometer.

2.5.3. Study design

Swiss albino mice were divided into seven groups (8 mice per group). At first, EST was established in 32 mice by intramuscular injection of 200 μL of cells (2.5×10^6 cells/mL) in the right thigh of mice[20]. On the 5th day after the EST inoculation, the mice were randomly divided into groups including non-EST mice receiving normal saline (C1), EST mice receiving normal saline (C2), EST mice treated with cyclophosphamide (CP) (5 mg/kg) intraperitoneally once a day for 14 d (C3), EST mice treated with PAME 50 mg/kg orally once a day for 2 weeks (Ex1), and EST mice treated with PAME 100 mg/kg orally once a day for 2 weeks (Ex2). The selection of doses of PAME was based on the pilot experiments and previous study[21]. Furthermore, two groups of healthy mice were used to evaluate the sub-acute toxicity of PAME including mice treated with PAME 50 mg/kg orally once a day for 2 weeks (C4), mice treated with PAME 100 mg/kg orally once a day for 2 weeks (C5)[13].

2.5.4. Blood sampling

At first, the mice were euthanized with ketamine (100 mg/kg) along with xylazine (10 mg/kg). Then, their abdomen cavities were opened. Blood samples were obtained from the heart of mice. After centrifuging the blood samples at 6000 rpm for 10 min, the obtained sera were separated and kept at −80 ℃ until examination.

2.5.5. Body weight changes

Mice were weighed on day 5 and day 19. The percentage of the obtained weight was assessed using the equation[21]: %weight gain = (Mice weight on 19th day/Mice weight on 5th day−1)×100.

2.5.6. Tumor sampling

After sterile aseptically removing of tumors in mice of C2, C3, Ex1, and Ex2 groups, they were weighed, and their dimensions were recorded. For further analysis, tumors were equally divided into two parts: the first part was kept at −80 ℃ for molecular tests, and the second part was kept at −20 ℃ for other tests.

2.5.7. Tumor growth inhibition

The antitumor activity of PAME was measured by calculating the tumor volume and tumor growth inhibition rate. Tumor volume was calculated by the vernier caliper after the 7th day of the treatment by the equation: tumor volume (mm^3) = $4\ (A/2)^2\ (B/2)$. So that, A and B are the small and big tumor axes. Tumor growth inhibition rate was determined based on the below formula:

Tumor growth inhibition rate (%)= (The mean tumor weight of control group−the mean tumor weight of treated group)/The mean tumor weight of control group C2×100[13].

2.5.8. Evaluation of the tumor markers

The level of alpha-fetoprotein (AFP) was determined using a mini-VIDAS® AFP kit based on the manufacturer's protocols. In addition, the serum level of carcinoembryonic antigen (CEA) was also evaluated by MyBio-Source Mice Carcinoembryonic Antigen Elisa Kit.

2.5.9. Evaluation of serum levels of liver enzymes

Serum levels of some liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in mice of all groups were measured using the commercial diagnostic kits[22].

2.5.10. Evaluation of serum levels of kidney enzymes

The level of serum creatinine (Cr) and blood urea nitrogen (BUN) were determined in mice of all groups using the commercial diagnostic kits[22].

2.6. Cellular mechanisms

2.6.1. Evaluation of the oxidative stress markers

To evaluate the lipid peroxidation, tumor homogenates were studied by biodiagnostic analysis kits based on the malondialdehyde (MDA) production using the thiobarbituric acid (TBA) methodology explained by of El-Aarag *et al.*[23]; in addition, NO production (Abcam, USA) was measured in the hepatic suspension technique defined by Green *et al.*[24]. Briefly, in this method, the thiobarbituric acid reacts with MDA in the liver homogenate to produce a thiobarbituric acid reactive product. Then, the absorbance was calculated at 534 nm.

2.6.2. Evaluation of the antioxidant enzymes

The levels of some enzymes involved in antioxidant activities such as glutathione peroxidase (GPx), catalase enzyme (CAT), and superoxide dismutase enzyme (SOD) activities were determined using the commercial kits and based on the method described by Weydert[25], El-Aarag[26], and Sun *et al.*[27], respectively.

2.6.3. Measurement of tumor necrosis factor–alpha (TNF–α) level

TNF-α levels in tumor homogenates were examined by the mice TNF-α ELISA kit based on the manufacturer's protocol.

2.6.4. Evaluation of the apoptosis–regulatory gene expression

The expression levels of some apoptosis-regulatory genes such as *caspase-3*, *Bcl-2*, and *Bax* were evaluated by the quantitative real-time PCR. In brief, the total RNA of tumor tissue was extracted by an RNeasy tissue kit based on the guidelines of the manufacturer. The cDNA was synthesized using the random primers according to the manufacturer's instructions. The synthesized cDNA was then used for real-time PCR by SYBR green. The thermal program of reaction was 96 ℃ for 7 min, 40 cycles of 95 ℃ for 10 s, and 56 ℃ for 30 s, respectively. Lastly, the $2^{-\triangle\triangle Ct}$ was calculated using the iQTM5 optical system software (Bio-Rad, Hercules, CA). The housekeeping gene was *β-actin*. Table 1 displays oligonucleotide primers which were used for real-time PCR[28].

2.7. Statistical analysis

The obtained data were presented as mean ± standard deviation. SPSS statistical software version, 22.0 (SPSS Inc., Chicago, IL, USA) was used for analysis. One-way ANOVA with Turkey's *post hoc* test was used to assess differences between experimental groups.

2.8. Ethical statement

This study was approved by the Ethical Committee of Scientific Research, Al-Quwayiyah, Shaqra University, Saudi Arabia (No. 20201123).

Table 1. The sequence of primers used for real-time PCR.

Amplicon	Primers sequence (5'–3')
Bax	F: GGCTGGACACTGGACTTCCT
	R: GGTGAGGACTCCAGCCACAA
Bcl–2	F: CATGCCAAGAGGGAAACACCAGAA
	R: GTGCTTTGCATTCTTGGA TGAGGG
Caspase–3	F: TTCATTATTCAGGCCTGCCGAGG
	R: TTCTGACAGGCCATGTCATCCTCA
β-actin	F: GTGACGTTGACATCCGTAAAGA
	R: GCCGGACTCATCGTACTCC

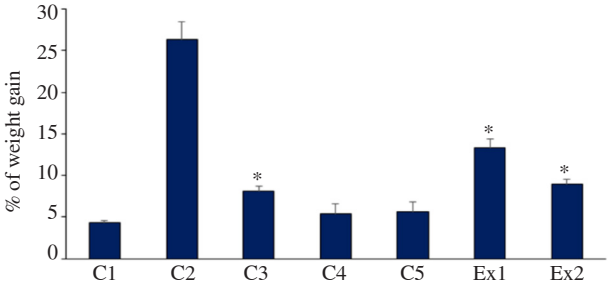

Figure 1. Mean of % of weight gains. C1: non-Ehrlich solid tumors (EST) and non-treated mice; C2: EST mice receiving normal saline; C3: EST mice treated with cyclophosphamide; C4: healthy mice receiving the PAME at a dose of 50 mg/kg; C5: healthy mice receiving the PAME at a dose of 100 mg/kg; Ex1: EST mice treated with PAME 50 mg/kg; Ex2: EST mice treated with PAME 100 mg/kg. Data are expressed as mean±SD. *: P<0.001, significant difference compared with the C2 group.

3. Results

3.1. Phytochemical analysis

The results exhibited the existence of a high volume of tannins, terpenoids, glycosides, and flavonoids as well as the lack of the alkaloids and saponins in the tested herb.

3.2. Secondary metabolites contents

The results showed that the total flavonoid, phenolic, and tannin content was 3.68 (mg QE/g DW), 74.6 (mg GEA/g DW), and 2.34 (mg CE/g DW), respectively.

3.3. Body weight evaluation

Figure 1 demonstrates that EST mice treated with CP and PAME at 50 and 100 mg/kg showed a significant decline in body weight (P<0.05) when compared with that of untreated EST mice in the C2 control group.

3.4. Tumor growth inhibition

Compared with the C2 group [(2.86±0.12) g], treatment wtih PAME significantly decreased the tumor volume in a dose-dependent manner in EST mice (P<0.05) [50 mg/kg: (1.93±0.07) g, 100 mg/kg: (1.12±0.05) g]. The tumor inhibition rate was 32.5% and 60.8% after treatment with PAME at 50 and 100 mg/kg, respectively.

3.5. Evaluation of tumor markers

The results revealed that in the C2 group, the serum level of CEA and AFP was significantly elevated compared with the C1 group (P<0.001). As shown in Figure 2, the level of CEA and AFP was significantly declined in mice bearing EST treated with CP and PAME at 50 and 100 mg/kg when compared with the mice of the C2 group (P<0.001).

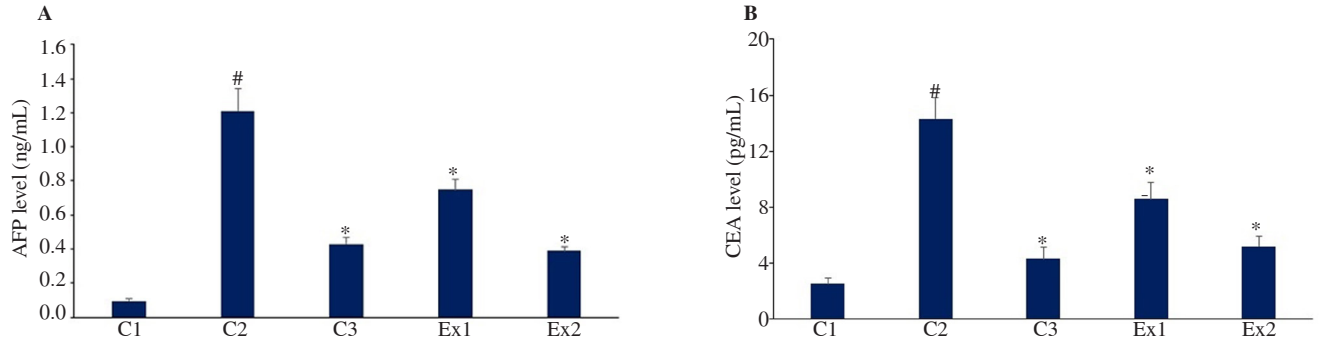

Figure 2. Serum level of alpha-fetoprotein (AFP) (A) and carcinoembryonic antigen (CEA) (B). Data are expressed as mean±SD. #P<0.001 significant difference compared with the C1 group; *P<0.001 significant difference compared with the C2 group.

3.6. Evaluation of serum level of liver enzymes

As shown in Figure 3, the serum level of AST and ALT was significantly increased in the control group of C2 (P<0.001); whereas CP and PAME at 50 and 100 mg/kg significantly reduced the level of ALT and AST (P<0.001) compared with the C2 group. The findings also exhibited that after the oral administration of healthy mice with PAME at 50 (C4) and 100 (C5) mg/kg for 14 d, the levels of AST and ALT showed no significant difference compared with the control group C1.

3.7. Evaluation of serum level of kidney enzymes

Figure 4 demonstrates that the serum level of BUN and Cr was significantly increased in the control group of C2; whereas CP and PAME at 50 and 100 mg/kg significantly reduced the level of BUN and Cr (P<0.001) compared with the mice of the C2 group. The findings also exhibited that after the oral administration of healthy mice with PAME at 50 (C4) and 100 (C5) mg/kg for 14 d, the level of BUN and Cr showed no significant difference in comparison with the control group C1.

3.8. Evaluation of the oxidative and antioxidant markers

As shown in Figure 5, the level of tumor MDA and NO was significantly elevated in the C2 group; while CP and PAME at 50 and 100 mg/kg/day significantly reduced MDA and NO (P<0.05). The level of GPx, CAT, and SOD was significantly declined in the C2 group. However, PAME at 50 and 100 mg/kg/day significantly increased the level of GPx, CAT, and SOD (P<0.05).

3.9. Measurement of the TNF–α level

The results exhibited that the level of TNF-α in mice of the C2 control group was significantly increased; whereas treatment with CP and PAME at 50 and 100 mg/kg/day significantly decreased the level of TNF-α in mice (P<0.05) (Figure 6).

3.10. Evaluation of the apoptosis–regulatory gene expression

The expression of the *caspase-3* gene was significantly up-regulated in tumor tissues (P<0.001), with 3.21-, 2.58- and 3.10-fold after treatment of CP and PAME at 50 and 100 mg/kg, respectively. The expression of the *Bax* gene was also significantly (P<0.001) up-regulated in tumor tissues (P<0.001), by 3.42-, 2.79- and 3.60- fold after treatment of CP and PAME at 50 and 100 mg/kg, respectively. The expression level of *Bcl-2* was downregulated after treatment of PAME.

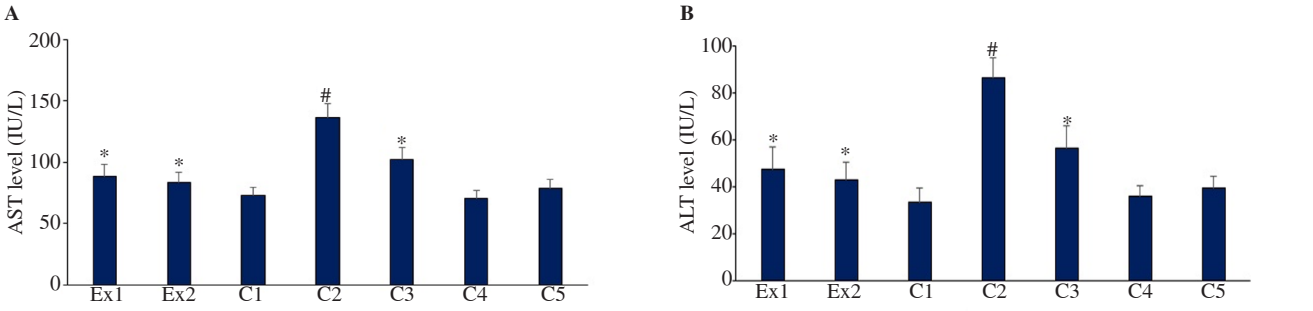

Figure 3. Serum level of (A) aspartate aminotransferase (AST) and (B) alanine aminotransferase (ALT). Data are expressed as mean±SD. #P<0.001 significant difference compared with the C1 group; *P<0.001 significant difference compared with the C2 group.

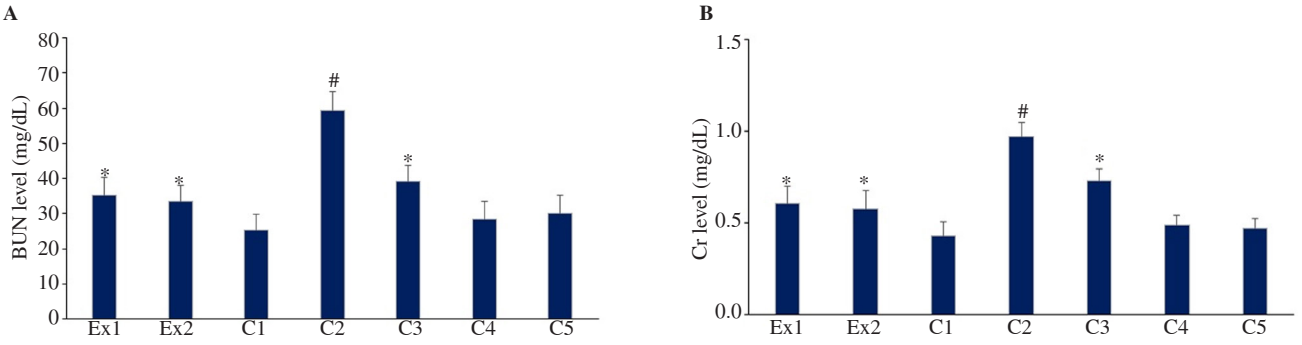

Figure 4. Serum level of (A) blood urea nitrogen (BUN) and (B) creatinine (Cr). Data are expressed as mean±SD. #P<0.001 significant difference compared with the C1 group; *P<0.001 significant difference compared with the C2 group.

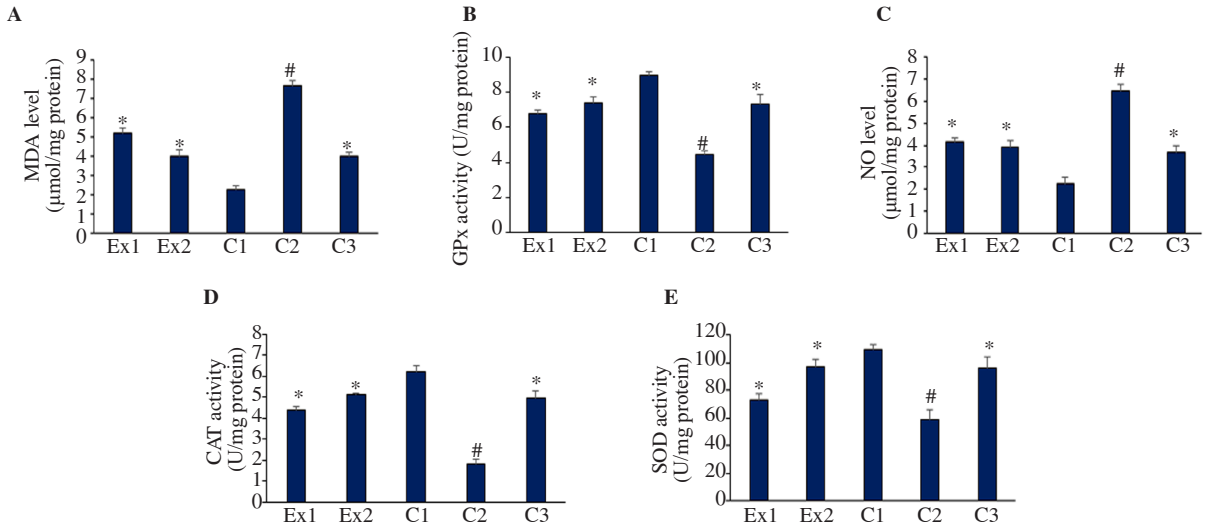

Figure 5. Tumor level of the malondialdehyde (MDA) and nitric oxide (NO), glutathione peroxidase (GPx), catalase enzyme (CAT), and superoxide dismutase enzyme activity (SOD). Data are expressed as mean±SD. #*P*<0.001 significant difference compared with the C1 group; *P<0.05 significant difference compared with the C2 group.

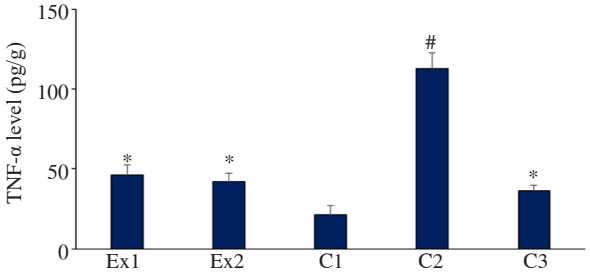

Figure 6. TNF-α level. Data are expressed as mean±SD. #*P*<0.001 significant difference compared with the C1 group; *P<0.05 significant difference compared with the C2 group.

4. Discussion

In many parts of the world, cancer is the 2nd principal reason for death after cardiovascular disease. Current problems in the use of chemotherapy and radiation therapy and the numerous side effects, as well as the resistance of cancer cells to common therapies, have led researchers to tend to new drugs with greater effectiveness and less toxicity[1–3]. Nature is an amazing source of suitable new drug compounds with great chemical diversity[29,30]. Therefore, in the present study, we aimed to evaluate the anti-tumor effects of PAME against the Ehrlich ascites carcinoma as a spontaneous breast carcinoma model in mice.

Our findings revealed that treatment of mice bearing EST with PAME at 50 and 100 mg/kg significantly decreased the tumor weight and volume. Moreover, tumor inhibition rate was 32.5% and 60.8%, respectively. Considering the anticancer activity of *P. atlantica*, Shafiei *et al*. have demonstrated cytotoxic effects of *P. atlantica* gum resin against human bile duct cancer (CC_{50} value of 15.3 μg/mL), pancreatic carcinoma (CC_{50} value of 11.5 μg/mL), gastric adenocarcinoma (CC_{50} value of 8.1 μg/mL), and COLO205 cells (CC_{50} value of 5.2 μg/mL)[12]. In the other study, Hashemi *et al*. have reported the cytotoxic effects of *P. atlantica* ethanolic extract against AGS (IC_{50} value of 382 μg/mL) and HeLa cells (IC_{50} value of 332 μg/mL)[31].

Today, serum levels of AFP and CEA are considered as the main tumor markers that become raised during some cancers development[32]. The elevation of these markers might indicate the liver and renal damage induced by the invasion of cancer cells. Our results demonstrated that the serum levels of CEA and AFP were significantly increased in the control group of C2, while PAME at 50 and 100 mg/kg significantly reduced the level of CEA and AFP (*P*<0.001).

Studies demonstrated that the cancer cells interrupted the metabolism of the normal liver cell which elevated the activity of serum enzymes, the destruction of hepatocytes by the invasion of cancer cells results in the release of AST and ALT into the plasma and subsequently the elevation of these liver enzymes[33]. In line with previous studies[34,35], we found that the serum level of AST and ALT was significantly increased in mice bearing EST, indicating that EST results in severe hepatocellular damage. However, treatment of PAME at 50 and 100 mg/kg significantly

reduced the level of ALT and AST (P<0.001).

Previous studies have demonstrated that EST impairs renal function and results in increasing blood BUN and Cr[36]. Similarly, we found that the serum level of BUN and Cr was significantly increased in mice bearing EST; whereas PAME at 50 and 100 mg/kg significantly reduced the level of BUN and Cr (P<0.001).

It has been proven that oxidative stress is mainly involved in the development of cancers and cardiovascular diseases[37]. Nowadays, studies demonstrated that antioxidants derived from natural products are potentially able to protect human beings from damages due to oxidative stress[38]. Our findings revealed that the levels of tumor MDA and NO were significantly elevated in the C2 group, which are significantly (P<0.05) reduced by PAME at 50 and 100 mg/kg. The levels of GPx, CAT, and SOD were significantly declined in the C2 group, which were significantly (P<0.05) increased by PAME at 50 and 100 mg/kg. In line with the current results, several studies reported the antioxidant effects of various parts of *P. atlantica* by DPPH-based radical scavenging, nitric oxide scavenging, β-carotene bleaching tests, *etc*[10,39]. Previous investigations have shown that the potent antioxidant effect of the plant is due to the presence of some phenolic (*e.g.* galloylquinic acid, quinic acid, gallic acid) and terpenes compounds whose antioxidant effects have already been proven[10,40].

Cancer cells can lead to serious damage as well as hypoxia in normal tissues, not only by physically damaging the cells but also by stimulating the production of some pro-inflammatory cytokines[41]. The results of the present study are similar to the previous study[42]. The level of pro-inflammatory cytokine TNF-α in EST mice was significantly increased; whereas treatment of PAME at 50 and 100 mg/kg significantly (P<0.05) decreased the level of TNF-α in mice. The main mechanism is its ability to scavenge free radicals as well as its anti-inflammatory effect[43].

Programmed-cell death which also called "apoptosis" is a form of cell death that occurs in response to various stressors such as physiological, pathological, or cytotoxic stimuli in the body. Apoptosis plays a role in different stages of a living organism's biological evolution and can cause various diseases if it is out of control[44]. One of the main processes in tumorigenesis and cancers is the inhibition of apoptosis which is vital for cancer cells to keep their uncontrollable multiplying. Consequently, induction of apoptosis is considered a main approach to treating cancer[45].

In this study, the expression level of some apoptotic genes (*caspase-3*, *Bcl-2*, and *Bax*) in tumor tissues was evaluated by quantitative real-time PCR. Our findings exhibited the expression of *caspase-3* and *Bax* gene was significantly increased in tumor tissues (P<0.001) after treatment of PAME at 50 and 100 mg/kg. In contrast, the expression level of *Bcl-2* was downregulated. Similarly, in the study conducted by Shafiei *et al*., *P. atlantica* gum resin extract showed their anticancer effects against KMBC, PANC-1, CRL-1739, and COLO205 cancer cells through the upregulation of *caspase-9*, *caspase-3*, and *Bax*, as well as the downregulation of *Bcl-2* genes[12]. Amiri *et al*. have also reported that *P. atlantica* ethanolic extract increased the expression of *Bax* and *caspase-3* genes and down-regulated the *Bcl-2* expression in human prostate cancer pc3 cells after 48 h exposure[11].

As a limitation of the study, we didn't separate the main and effective compounds of the plant that may have anti-cancer effects or explore other possible anti-cancer mechanisms, neither, which will be studied in our further experiment.

In conclusion, our study revealed that *P. atlantica* methanolic extract could reduce tumor volume, body weight, tumor markers (AFP and CEA), serum level of liver and kidney enzymes, MDA, NO, and TNF-α level; whereas PAME at 50 and 100 mg/kg/day significantly increased the level of antioxidant enzymes of GPx, CAT, and SOD in EST mice. Therefore, the extract might be considered as an alternative anticancer agent against tumors, however, additional studies especially in the clinical setting are required to confirm this finding.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

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Authors' contributions

AEA and NAA contributed in study design, conceptualization, methodology, and investigation. RHA and SFA performed the experiments and analyzed data. AEA, SFA and NAA supervised the study and wrote the original draft of the manuscript. All authors have read and approved the final manuscript.

References

[1] Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**(1): 57-70.

[2] Zhou H, Zou P, Chen ZC, You Y. A novel vicious cycle cascade in tumor chemotherapy. *Med Hypotheses* 2007; **69**(6): 1230-1233.

[3] Chabner BA, Roberts TG. Chemotherapy and the war on cancer. *Nat Rev Cancer* 2005; **5**(1): 65-72.

[4] Diaby V, Tawk R, Sanogo V, Xiao H, Montero AJ. A review of systematic reviews of the cost-effectiveness of hormone therapy,

chemotherapy, and targeted therapy for breast cancer. *Breast Cancer Res Treat* 2015; **151**(1): 27-40.

[5] Valenti RB. Chemotherapy education for patients with cancer: A literature review. *Clin J Oncol Nurs* 2014; **18**(6): 637.

[6] Joyce Nirmala M, Samundeeswari A, DeepaSankar P. Natural plant resources in anti-cancer therapy-A review. *Res Plant Biol* 2011; **1**(3): 1-14.

[7] Agarwal N, Majee C, Chakraborthy GS. Natural herbs as anticancer drugs. *Int J PharmTech Res* 2012; **4**(3): 1142-1153.

[8] Bozorgi M, Memariani Z, Mobli M, SalehiSurmaghi MH, Shams-Ardekani MR, Rahimi R. Five *Pistacia* species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*): A review of their traditional uses, phytochemistry, and pharmacology. *Sci World J* 2013; **2013**: 219815.

[9] Ahmed ZB, Yousfi M, Viaene J, Dejaegher B, Demeyer K, Vander Heyden Y. Four *Pistacia atlantica* subspecies (*atlantica*, *cabulica*, *kurdica* and *mutica*): A review of their botany, ethnobotany, phytochemistry and pharmacology. *J Ethnopharmacol* 2020; **265**: 113329.

[10]Mahjoub F, Rezayat KA, Yousefi M, Mohebbi M, Salari R. *Pistacia atlantica* Desf. A review of its traditional uses, phytochemicals and pharmacology. *J Med Life* 2018; **11**(3): 180.

[11]Amiri M, Kazerouni F, Namaki S, Tamijani HD, Rahimipour H, Boroumand N, et al. Cytotoxic effects of the ethanol bane skin extract in human prostate cancer Pc3 cells. *Iran J Cancer Prev* 2016; **9**(2): e4755.

[12]Shafiei BH, Shasaltaneh MD, Ghaffari SM, Ahmadian S, Kamarehei M, Raizi GH. An *in vitro* study on the effect of *Pistacia atlantica* sub kurdica extract on microtubule proteins: A potential anti-cancer compound. *Int J Pharm Sci Res* 2015; **6**(12): 5029.

[13]Almeer RS, Aref AM, Hussein RA, Othman MS, Abdel Moneim AE. Antitumor potential of berberine and cinnamic acid against solid Ehrlich carcinoma in mice. *Anticancer Agents Med Chem* 2019; **19**(3): 356-364.

[14]Ozaslan M, Karagoz ID, Kilic IH, Guldur ME. Ehrlich ascites carcinoma. *Afr J Biotech* 2011; **10**(13): 2375-2378.

[15]Ezatpour B, Saedi Dezaki E, Mahmoudvand H, Azadpour M, Ezzatkhah F. *In vitro* and *in vivo* antileishmanial effects of *Pistacia khinjuk* against *Leishmania tropica* and *Leishmania major*. *Evid Based Complement Alternat Med* 2015; **2015**: 149707.

[16]Nazer MR, Jahanbakhsh S, Ebrahimi K, Niazi M, Sepahvand M, Khatami M, et al. Cytotoxic and antileishmanial effects of various extracts of *Capparis spinosa* L. *Turkish J Pharm Sci* 2021; **18**(2): 146.

[17]Singleton VL, Orthofer R, Lamuela-Raventó RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol* 1999; **299**: 152-178.

[18]Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *Sci World J* 2020; **2020**: :8780704.

[19]Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. *J Sci Food Agric* 1978; **29**(9): 788-794.

[20]El-Aarag BY, Kasai T, Zahran MA, Zakhary NI, Shigehiro T, Sekhar SC, et al. *In vitro* anti-proliferative and anti-angiogenic activities of thalidomide dithiocarbamate analogs. *Int Immunopharmacol* 2014; **21**(2): 283-292.

[21]Mahmoudvand H, Kheirandish F, Ghasemi Kia M, Tavakoli Kareshk A, Yarahmadi M. Chemical composition, protoscolicidal effects and acute toxicity of *Pistacia atlantica* Desf. fruit extract. *Nat Prod Res* 2016; **30**(10): 1208-1211.

[22]Jaganathan SK, Mondhe D, Wani ZA, Pal HC, Mandal M. Effect of honey and eugenol on Ehrlich ascites and solid carcinoma. *J Biomed Biotechnol* 2010; **2010**: 989163-989165.

[23]El-Aarag B, Kasai T, Masuda J, Agwa H, Zahran M, Seno M. Anticancer effects of novel thalidomide analogs in A549 cells through inhibition of vascular endothelial growth factor and matrix metalloproteinase-2. *Biomed Pharmacother* 2017; **85**: 549-555.

[24]Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal Biochem* 1982; **126**: 131-138.

[25]Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nat Protoc* 2010; **5**(1): 51-66.

[26]El-Aarag B, Magdy M, AlAjmi MF, Khalifa SAM, El-Seedi HR. Melittin exerts beneficial effects on paraquat-induced lung injuries in mice by modifying oxidative stress and apoptosis. *Molecules* 2019; **24**(8): 1498.

[27]Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988; **34**(3): 497-500.

[28]Selim NM, Elgazar AA, Abdel-Hamid NM, El-Magd MRA, Yasri A, Hefnawy HME, et al. Chrysophanol, physcion, hesperidin and curcumin modulate the gene expression of pro-inflammatory mediators induced by LPS in hepg2: *In silico* and molecular studies. *Antioxidants* 2019; **8**: 371.

[29]Venugopala KN, Rashmi V, Odhav B. Review on natural coumarin lead compounds for their pharmacological activity. *Biomed Res Int* 2013; **2013**: 963248.

[30]Kaefer CM, Milner JA. The role of herbs and spices in cancer prevention. *J Nutr Biochem* 2008; **19**(6): 347-361.

[31]Hashemi L, Asadi-Samani M, Moradi MT, Alidadi S. Anticancer activity and phenolic compounds of *Pistacia atlantica* extract. *Int J Pharm Phytopharmacol Res* 2017; **7**(2): 26-31.

[32]Kim JH, Jun KH, Jung H, Park IS, Chin HM. Prognostic value of preoperative serum levels of five tumor markers (Carcinoembryonic antigen, CA19-9, Alpha-fetoprotein, CA72-4, and CA125) in gastric cancer. *Hepatogastroenterology* 2014; **61**(131): 863-869.

[33]Saravanan R, Viswanathan P, Pugalendi KV. Protective effect of ursolic acid on ethanol-mediated experimental liver damage in rats. *Life Sci* 2006; **78**(7): 713-718.

[34]Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamsi ML.

Antitumor activity and antioxidant status of *Caesalpinia bonducella* against Ehrlich ascites carcinoma in Swiss albino mice. *J Pharmacol Sci* 2004; **94**(2): 177-184.

[35]Abou Zaid OA, Hassanein MR, EL-Senosi YA, EL-Shiekha MF. Biochemical effect of some antioxidant on metabolic changes in experimentally induced tumor in female mice. *Benha Veter Med J* 2011; **1**(1): 52-60.

[36]Hussein SA, Azab ME. Effect of insulin treatment on some metabolic changes on experimentally induced tumor in female mice. *Egypt J Biochem Mol Biol* 1997; **15**: 61-80.

[37]Visconti R, Grieco D. New insights on oxidative stress in cancer. *Curr Opin Drug Disc Devel* 2009; **12**(2): 240-245.

[38]Chen W, Jia Z, Pan MH, Anandh Babu PV. Natural products for the prevention of oxidative stress-related diseases: Mechanisms and strategies. *Oxid Med Cell Long* 2016; **2016**: 4628502.

[39]Toul F, Belyagoubi-Benhammou N, Zitouni A, Atik-Bekkara F. Antioxidant activity and phenolic profile of different organs of *Pistacia atlantica* Desf. subsp. *atlantica* from Algeria. *Nat Prod Res* 2017; **31**(6): 718-723.

[40]Ben Ahmed Z, Yousfi M, Viaene J, Dejaegher B, Demeyer K, Mangelings D, et al. Seasonal, gender and regional variations in total phenolic, flavonoid, and condensed tannins contents and in antioxidant properties from *Pistacia atlantica* ssp. leaves. *Pharm Biol* 2017; **55**(1): 1185-1194.

[41]Balkwill F. TNF-α in promotion and progression of cancer. *Cancer Metastasis Rev* 2006; **25**(3): 409-416.

[42]El-Dayem SM, Fouda FM, Ali EH, Motelp BA. The antitumor effects of tetrodotoxin and/or doxorubicin on Ehrlich ascites carcinoma-bearing female mice. *Toxicol Ind Health* 2013; **29**(5): 404-417.

[43]Shen J, Xiao Z, Zhao Q, Li M, Wu X, Zhang L, et al. Anti-cancer therapy with TNFα and IFNγ: A comprehensive review. *Cell Prolif* 2018; **51**(4): e12441.

[44]Letai A. Apoptosis and cancer. *Ann Rev Cancer Biol* 2017; **1**: 275-294.

[45]Koff JL, Ramachandiran S, Bernal-Mizrachi L. A time to kill: Targeting apoptosis in cancer. *Int J Mol Sci* 2015; **16**(2): 2942-2955.