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ORIGINAL PAPER

Antitumor effects of Dadas Cress (*Lepidium sativum* var. *sativum*) on Ehrlich Ascites tumor cells: an in vitro and in vivo study

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Abstract

Garden cress (Lepidium sativum L.) is widely used in nutrition and traditional medicine for its bioactive properties. Studies show its seeds and leaves have anticancer, antimicrobial, and antidiabetic effects. This study investigated the antitumor potential of an extract from the leaves of Dadaş cress (*Lepidium sativum* var. *sativum*), a Turkish variety, against Ehrlich Ascites Tumor (EAT) cells. In the in vitro study, Dadaş cress extract (DCE) was tested at 25, 50, and 100 µg/mL concentrations to evaluate its antitumor activity. Caspase-3/7 activity was measured by fluorometric assay, mitochondrial membrane depolarization by JC-1 dye, and cell cycle by flow cytometry. The 50 µg/mL group had the highest apoptosis rate at 48 h; 100 µg/mL caused the most mitochondrial depolarization at 24 h. After 72 h, the 5-FU group had the highest G0/G1 phase cells, while the 25 µg/mL DCE group had the highest S phase cells. In vivo, groups were control, EAT control, EAT + 5-FU, EAT + DCE (75–150 mg/kg), and DCE only (75–150 mg/kg). Liver and kidney tissues were examined immunohistochemically, biochemically, and genotoxically. DCE significantly lowered TNF-α expression, oxidative stress, and DNA damage in EAT mice. In the 150 mg/kg DCE group, renal tail DNA% dropped from 92.5 to 34.8%, liver tail DNA% from 105.3 to 65.8%. TAS increased, TOS decreased vs. EAT control ($p < 0.05$). These results suggest DCE protects against EAT-induced damage dose-dependently and has no genotoxicity. The findings suggest that DCE may have antitumor potential.

Keywords Antitumor effect · Apoptosis · EAT · Garden cress

Introduction

One of the major obstacles in cancer treatment is chemotherapy resistance, which arises from the biological heterogeneity of tumors and limits treatment response. This resistance leads to disease progression and treatment failure through molecular mechanisms that vary between patients, complicating clinical management (Vasan et al. 2019). Chemotherapy-induced drug resistance is frequently associated with the upregulation of resistance genes such as MDR1; various factors, including mutational changes, epigenetic mechanisms, activation of the Wnt signaling pathway, and loss of p53 function, regulate this process. In particular, in p53 mutation-related chemotherapy resistance, suppression of miR-34a expression results in the accumulation of LRPPRC protein, which stabilizes MDR1 mRNA, thereby increasing MDR1 expression and facilitating drug efflux from the cell (Banerjee et al., 2016; Yang et al. 2022).

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These mechanisms highlight the need for novel therapeutic approaches to overcome such resistance.

Given the limitations and adverse effects of conventional chemotherapeutics, there is a growing interest in plant-derived compounds with pharmacological potential. Several studies have demonstrated the anticancer effects of these phytochemicals on a wide range of cancer cell lines (Vanduchova et al. 2019; Yilmaz et al. 2020). A significant portion of the global population reports using complementary and alternative therapies in the context of cancer (Shin et al. 2012; Yarney et al. 2013; Dhano et al., 2014; Naja et al. 2015; Wode et al. 2019; Misawa et al. 2019). This trend underscores the relevance of scientifically investigating the therapeutic role of medicinal plants in cancer prevention and treatment.

Among the most widely studied mechanisms through which phytochemicals exert anticancer effects is apoptosis, a regulated cell death. Apoptosis can be triggered by DNA damage, elevated intracellular calcium levels, hypoxia, activation of death receptors (e.g., Fas, TNF), and chemotherapeutic agents (Pucci et al. 2000; Kaczanowski 2016; Reyes-Fermin et al. 2020; Tang et al. 2020). A key event in this process is the reduction of mitochondrial membrane potential, which leads to caspase activation and proteolytic degradation of cellular components (Lu et al. 2018). Notably, while tumor necrosis factor-alpha (TNF-α) has antineoplastic properties, its secretion by host immune cells in the tumor microenvironment can paradoxically promote tumor progression and inflammation (Zhou et al., 2017; Sheng et al. 2018; Cruceriu et al. 2019).

Phytochemicals—especially phenolic compounds, flavonoids, and glucosinolate derivatives—are known for modulating these pathways. They exert antioxidant, antiproliferative, and proapoptotic effects in cancer models (Mathur et al. 2015; Peters & Gonzales, 2018; Mitsiogianni et al. 2021; Klaunig 2018). Phenolic compounds and flavonoids have been shown to inhibit cancer cell proliferation, induce apoptosis, and suppress metastasis (Kopustinskiene et al. 2020; Sharma et al. 2022). Specifically, flavonoids such as quercetin and rutin have demonstrated tumor growth inhibition and cell cycle modulation in liver, breast, and colon cancers (Ghanbari-Movahed et al. 2022). In parallel, benzyl isothiocyanate (BITC), a hydrolysis product of glucosinolates, induces apoptosis via DNA damage and prevents tumor development (Keum et al. 2004; Xiao et al. 2008; Wu et al. 2009; Han et al. 2019).

In this context, Lepidium sativum (garden cress, GC), a traditionally used medicinal herb in Asia and Africa, has emerged as a promising phytochemical source. Both its leaves and seeds are rich in minerals (Ca, Mg, P, K, Cu, Mn) and vitamins (A, B6, C, K, riboflavin, folate) (Juma 2007; Diwakar et al. 2010; Sat et al. 2013; Chatoui et al. 2016). Notably, GC contains high levels of phenolics, flavonoids, and glucosinolates—especially BITC and related isothiocyanates. GC–MS analyses of its seed and leaf confirm the presence of these compounds, suggesting its strong bioactive potential. (Sarikamiş & Yanmaz, 2011; Alotaibi et al. 2021; Painuli et al. 2022).

Experimental studies further support the anticancer efficacy of GC. Both in vitro and in vivo assays have shown that GC extracts can enhance apoptosis, suppress proliferation, and inhibit tumor growth (Hekmatshoar et al. 2022). Combining its diverse phytochemical components may produce synergistic effects, strengthening its anticancer properties. This synergy is particularly evident in studies where GC inhibited tumor growth and induced cell death in breast (MCF-7), liver (HEPG2), and Ehrlich ascites carcinoma models (Painuli et al. 2022; Hekmatshoar et al. 2022).

The Ehrlich Ascites Tumor (EAT) model was chosen in the current study due to its well-established utility in cancer research. It is characterized by rapid and consistent tumor growth, high proliferation, and practical applicability in both in vitro and in vivo systems—without the need for immunosuppression. These features make EAT an ideal model for evaluating the antitumor efficacy of phytochemical-rich herbal preparations such as L. sativum (Sharada et al. 1996; Sharma et al., 2001; Tayel et al. 2021). In this study, we aimed to assess the anticancer activity of L. sativum var. sativum, an endemic Turkish variety of GC, using the EAT model. The findings suggest that GC may have potential for consideration in cancer research.

Method

In the present study, the applications performed on experimental animals were evaluated regarding animal rights and welfare, and their suitability in terms of animal experiments ethics was approved by the Erciyes University, Experimental Animals Local Ethics Committee, decision number 19/174 dated 09.10.2019. Experimental stages of the study were carried out at the Betül – Ziya EREN Genome and Stem Cell Center.

The extract and contents

L. Sativum var. *Sativum* extract

Lepidium sativum var. sativum seeds were obtained from Erzurum Atatürk University Faculty of Agriculture and cultivated. The plant was harvested in May in the Kayseri region of Turkey. The optimal temperature for seed germination is between 24 and 25 °C. The plant becomes ready for harvest approximately 28 days after sowing. The ideal

harvesting time is when the plant reaches a 25–30 cm height and develops a bushy structure with branches extending from the stem. The harvested plant was dried at approximately 24 °C in an environment free of sunlight and airflow. It was then ground into a fine powder. A magnetic stirrer mixed 100 g of the powdered material with 1 L of water at 60 °C for 6 h. At the end of the process, the mixture was filtered, lyophilized at 50 °C, and stored at − 20 °C. The extract was diluted with distilled water (Bağcı et al., 2022). Before application, the extract was freshly dissolved in distilled water at room temperature and kept at 4 °C until use. This procedure was consistently applied throughout the study to ensure reproducibility of the experiments. The solution was prepared at a concentration of 5 mg/mL. It was sequentially filtered through sterile 0.45 µm and 0.22 µm filters.

Total phenolic, flavonoid, and BITC contents

The total amount of phenol contained in the extracts was calculated as gallic acid equivalent using the Folin-Ciocalteu method (Singleton et al. 1999). The basis of this method is the reaction of phenolic compounds with the Folin-Ciocalteau (phosphomolybdic-phosphotungstic acid) reagent in an essential medium. The flavonoid total was calculated as catechin equivalent (Zhishen et al. 1999). The amount of BITC in DCE was determined using a High-Performance Liquid Chromatography (HPLC) system. (Kirchhoffer et al. 2023).

Formation of the stock mice

EAT cells used in this study were derived from a previously established in vivo stock tumor model, then frozen in cryotubes with 10% DMSO and stored at − 80 °C. Cells were thawed before use and injected intraperitoneally into stock animals for tumor propagation. The viability was checked on a Thoma slide after the stock cells were thawed at room temperature. 0.1 mL of EAT cells was injected intraperitoneally with an insulin injector. An ascitic tumor formed in the abdomen of the stock mouse after 7 days. EAT cells were used in both in vitro and in vivo studies.

In vitro study

Cell culture

The medium was prepared using Dulbecco's Modified Eagle Medium (DMEM), supplemented with 15% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco™ DMEM, manufactured at a cGMP-compliant facility in Paisley, Scotland, UK; Catalog no: 41966029). Cell viability was assessed qualitatively through microscopic observation for preliminary tests. Cells were examined under a light microscope to estimate the proportion of live and dead cells based on morphological characteristics and confluence. It provided sufficient preliminary information to select appropriate doses for subsequent experiments. The experimental groups were organized as follows: the control group (A), the 5-fluorouracil (5-FU) group at 15 µg/mL (B), and the groups treated with DCE at doses of 25 µg/mL (C), 50 µg/mL (D), and 100 µg/mL (E) to evaluate its antitumor activity on EAT cells. The extract was dissolved in DMEM. The solution was prepared at a concentration of 5 mg/mL. EAT cells were incubated at 37 °C, 95% humidity, and 5% CO2 for 24, 48, and 72 h (Yilmaz et al. 2023). At the end of each incubation period, the contents of each well were transferred to Eppendorf tubes for further analysis.

Mitopotential assay

The tubes were centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. After washing with PBS, the protocol for the kit (The Muse® MitoPotential Kit–100 Tests (Part Number: MCH100110) was applied. Samples were analyzed automatically with the Muse® Cell Analyzer (Merck-Millipore) according to the manufacturer's instructions (Haznedar et al. 2024).

Caspase-3/7 assay

The tubes were centrifuged at 2400 rpm for 5 min, and the supernatant was discarded. After washing with PBS, the protocol for the kit (The Muse® Caspase-3/7 Kit–100 Tests (Part Number: MCH100108) was applied (Kwak et al. 2021). The kit determines the count and percentage of cells in various stages of apoptosis based on the activity of Caspase-3/7. Samples were analyzed automatically with the Muse® Cell Analyzer (Merck-Millipore) according to the manufacturer's instructions.

Cell cycle assay

The tubes were centrifuged at 2400 rpm for 5 min, and the supernatant was discarded. After washing with PBS, the kit protocol was applied (The Muse® Cell Cycle Kit–100 Tests (Part Number: MCH100106). Samples were analyzed automatically with the Muse® Cell Analyzer (Merck-Millipore) according to the manufacturer's instructions (Bavelloni et al. 2017).

In vivo study

Design of the experimental groups

Male Balb/c mice, aged 8–10 weeks and weighing 25–30 g, were used in this study. The mice were housed five per cage in specially prepared, automatically air-conditioned chambers maintained at a constant temperature of 21 °C, with 12-hour light/dark cycles. Standard pellet feed and tap water were provided ad libitum. For tumor induction, 0.1 mL of EAT fluid containing 10^6 cells was injected intraperitoneally. During group allocation, animals were randomly assigned to treatment groups, and all interventions were conducted using within-group blinding. 5-FU was dissolved in saline prior to administration. All treatments were administered intraperitoneally. At the end of the experiment, the animals were sacrificed under general anesthesia using ketamine (50 mg/kg) and xylazine (15 mg/kg). Kidney and liver tissues were collected for the analysis.

Experimental Groups ($n = 10$ per group):

Control Group: Healthy mice received 0.1 mL of distilled water intraperitoneally once daily for 10 days.

EAT Control Group: Tumor-bearing mice received 0.1 mL of distilled water intraperitoneally once daily for 10 days.

EAT + 5-FU Group: Tumor-bearing mice were treated with 5-fluorouracil (5-FU) at 20 mg/kg/day intraperitoneally for 10 days.

EAT + 75 mg/kg DCE Group: Tumor-bearing mice were treated with DCE at a dose of 75 mg/kg/day intraperitoneally for 10 days.

EAT + 150 mg/kg DCE Group: Tumor-bearing mice were treated with DCE at a dose of 150 mg/kg/day intraperitoneally for 10 days.

75 mg/kg DCE Group: Healthy mice (no tumor induction) were treated with DCE at a dose of 75 mg/kg/day intraperitoneally for 10 days.

150 mg/kg DCE Group: Healthy mice (no tumor induction) were treated with DCE at a dose of 150 mg/kg/day intraperitoneally for 10 days.

Immunohistochemical analysis

Immunoreactivity TNF-α, in liver and kidney tissues, was determined using the Avidin-Biotin peroxidase method (Doğanyiğit et al. 2020). In summary, 5 µm thick sections were deparaffinized, and citrate buffer (pH 6.0; Thermo Fisher Scientific, UK, AP-9003-500) was used for epitope retrieval. The slides were then incubated in 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Ultra V Block solution (Thermo Fisher Scientific, UK, TA-125-UB) was applied to prevent non-specific staining. The sections were subsequently incubated overnight at 4 °C with TNF-α primary antibody (1:100 dilution; Elabscience, USA, E-AB-22159). After several washes with PBS, the sections were incubated for 40 min at 37 °C in an incubator with biotinylated goat anti-polyvalent secondary antibody (Thermo Fisher Scientific, UK, TP-125-BN). The immune reaction was visualized using diaminobenzidine (DAB) chromogen (Thermo Fisher Scientific, UK, TA-125-HD). Counterstaining was performed using Gill's hematoxylin (Merck, Germany, 1.05174.1000). The sections were dehydrated through a graded alcohol series and mounted with a mounting medium known as Entellan. Finally, the sections were examined under an Olympus BX53 light microscope. Immunoreactivity levels were evaluated using the ImageJ software (Version 1.46, National Institutes of Health, Bethesda, Maryland).

TAS-TOS analysis

Liver and kidney tissues from subjects were raised to − 80 °C. The tissues were homogenized before the study. Then, centrifugation was performed and supernatants were transferred to Eppendorf tubes for analysis (Kaymak et al. 2021). By studying with the enzyme-linked immunosorbent assay (ELISA) method following the Total Antioxidant Status (TAS) (Rel Assay Diagnostics, Turkey) and Total Oxidant Status (TOS) (Rel Assay Diagnostics, Turkey) kits. The quantities were determined using an ELISA reader: TAS at 660 nm in mmol/L and TOS at 530 nm in µmol/L.

Comet test analysis

The cells were mixed with low-melting-point agarose (0.65%), and 75 µL of the suspension was layered onto microscope slides precoated with normal-melting-point agarose (0.65%). A cover glass was placed over the suspension, and the slides were chilled at + 4 ◦C until solidified. Once solidified, the cover glass was removed, and the slides were immersed in a lysing solution. Subsequently, the slides were transferred to a gel electrophoresis platform containing electrophoresis buffer and incubated for 20 min to allow DNA unwinding (Baş et al. 2015). After incubation, the slides were transferred to a neutralizing tank and washed with neutralizing buffer. Ethidium bromide was applied directly onto the slides, and a cover glass was placed over them. Data analysis was performed using BS 200 ProP software (BAB Bs Comet Assay software) for image analysis (BS 200 ProP, BAB Imaging System, Ankara, Turkey).

Table 1 Total phenolic and flavonoid contents of *L. Sativum* var. *Sativum e*xtract

Extract	Total phenol [$mg_{GAE}/g_{extract}$]	Total flavonoid [$mg_{CA}/g_{extract}$]	Total BITC (mg/L)
Lepidium sativum var. *sativum*	159,62 ± 2,07	97,28 ± 4,35	1.787

GAE: Gallic acid equivalents; CA: Catechin equivalents

Statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences software, version 26.0 for Windows (SPSS, Chicago, IL,). The Shapiro–Wilk test was used to assess the normality of in vitro data, while the Kolmogorov–Smirnov test was employed for in vivo data. Additionally, histogram and Q–Q plots were examined to support the assessment of data distribution in both cases. For the in vitro experiments, the non-parametric Kruskal–Wallis H test was applied, and the results were expressed as median (minimum–maximum). The in vivo data were analyzed using parametric one-way analysis of variance (ANOVA). Homogeneity of variances was evaluated using Levene's test. Post hoc comparisons were performed using Tukey's test when significant differences were detected. Results were expressed as mean ± standard deviation (Mean ± SD). A p-value of < 0.05 was considered statistically significant.

Result

Total phenolic, flavonoid, and BITC contents

The total phenolic, flavonoid, and BITC contents of *L. sativum* var. *sativum* extract are shown in Table 1.

In vitro results

Mitopotential assay

The rate of live cells with depolarized membranes was statistically significant among doses after 24 h of incubation, with the highest rate observed in the 100 µg/ml DCE group ($p < 0.05$). At 48 and 72 h, the effects of DCE were limited or not significant compared to earlier time points (Fig. 1) (Table 2).

$p < 0.05$ was considered statistically significant. Data are expressed as median (min-max). There is no statistically significant difference between the groups containing the same letter ($^{a, b,c}$) ($p > 0,05$)

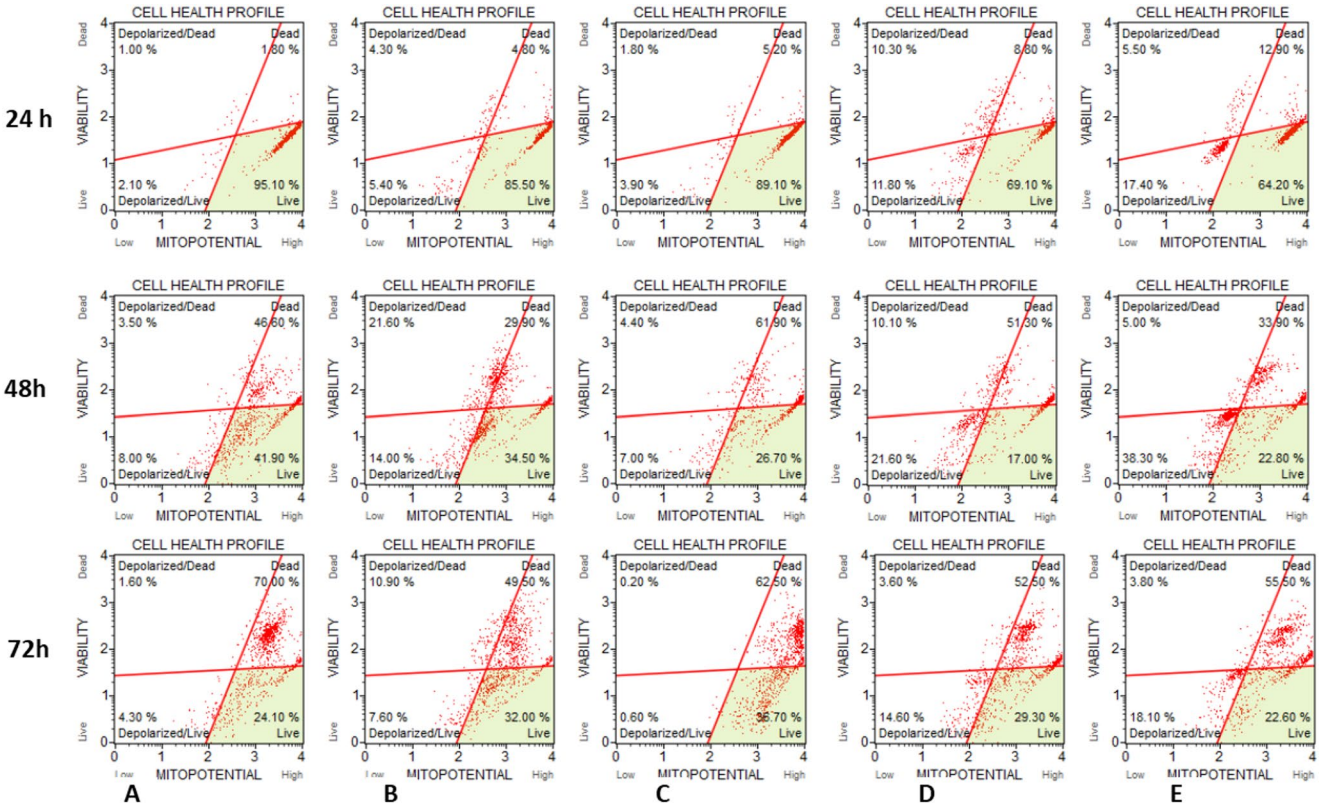

Fig. 1 Mitopotential assay: Data plots were obtained from the Muse Cell Analyzer after 24, 48, and 72 h of incubation. **A** EAT Control group, **B** EAT + 5-FU group, **C** EAT + 25 µg/ml DCE group, (D) EAT + 50 µg/ml DCE group, (E) EAT + 100 µg/ml DCE group

Table 2 Depolarized live EAT cells data (%) (24, 48, and 72 h incubation)

Hours /Groups	EAT Control (A)	EAT + 5-FU (B)	EAT + 25 µg/ml DCE (C)	EAT + 50 µg/ml DCE (D)	EAT + 100 µg/ml DCE (E)	*p*
24 h	2,86 (2,1–4)[a]	5,4 (4,8–10,5)[abc]	2,6 (2,4 − 3,9)[ab]	7,4 (3,7–11,8)[abc]	18,6 (17,4–22,7)[c]	0,026*
48 h	0 (0–8)	14 (8,6–14,4)	0,2 (0–7)	21,6 (10,7–26,6)	19,20 (6,3–38,3)	0,057
72 h	3 (0,1–4,3)	7,6 (1,5–9,9)	2,2 (0,6 − 4,7)	14,6 (6,5–19,4)	18,1 (0,7–22,7)	0,209

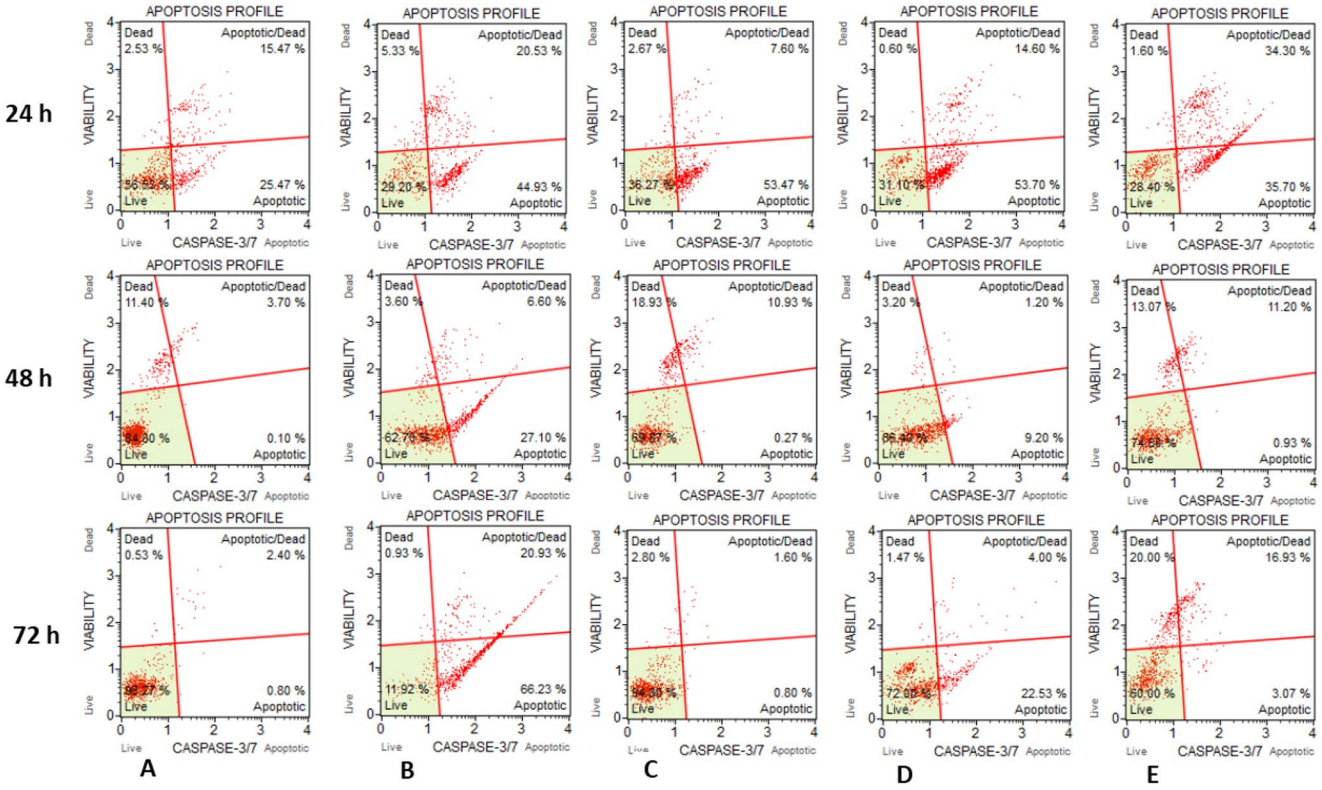

Fig. 2 Caspase-3/7 assay; Data plots were obtained from the Muse Cell Analyzer after 24, 48, and 72 h of incubation. **A** EAT Control group, **B** EAT + 5 FU group, **C** EAT + 25 µg/ml DCE group, **D** EAT + 50 µg/ml DCE group, **E** EAT + 100 µg/ml DCE group

Table 3 Total apoptotic EAT cell data (%) (24, 48 and 72 h incubation)

Hours /Groups	EAT Control (A)	EAT + 5-FU (B)	EAT + 25 µg/ml DCE (C)	EAT + 50 µg/ml DCE (D)	EAT + 100 µg/ml DCE (E)	*p*
24 h	13,65 (3,8–19,7)	1,37 (0,8–33,7)	2,8 (2–11,2)	2,67 (1,47 − 10,4)	12,13 (3,3–13,3)	0,367
48 h	3,07 (0–3,2)[a]	14,27(13,8–87,2)[a]	1,73 (1,07 − 2,4)[a]	26,53(3,8–50,8)[b]	15,47 (3,07 − 2)[a]	0,047*
72 h	19,33 (12,7–40,9)	65,47 (15,9–89,7)	61,07(2,8–89,5)	68,3 (10,1–79)	36,8 (27,3–70)	0,815

$p < 0.05$ was considered statistically significant. Data are expressed as median (min-max). There is no statistically significant difference between the groups containing the same letter ([a, b,c]) ($p > 0,05$)

Caspase-3/7 test

The total apoptotic cell rate was the highest in the group treated with 50 µg/ml DCE after 48 h of incubation, and it was statistically significant (p<0,05) (Fig. 2) (Table 3).

Cell cycle assay

At 72 h of incubation, there were statistically significant differences between groups in the G0/G1 and S phases ($p < 0.05$). In the G0/G1 phase, the group treated with 5-FU differed significantly, showing a higher proportion of cells. This suggests that 5-FU may induce cell cycle arrest or delay in the G0/G1 phase. In the S phase, the group treated with 25 µg/ml DCE showed a significantly higher cell proportion than other groups. This indicates that DCE at this dose may cause cell accumulation in the S phase. No statistically significant differences were observed among the groups at other time points and in the G2/M phase (Fig. 3) (Table 4).

$p < 0.05$ was considered statistically significant. Data are expressed as median (min-max). There is no statistically

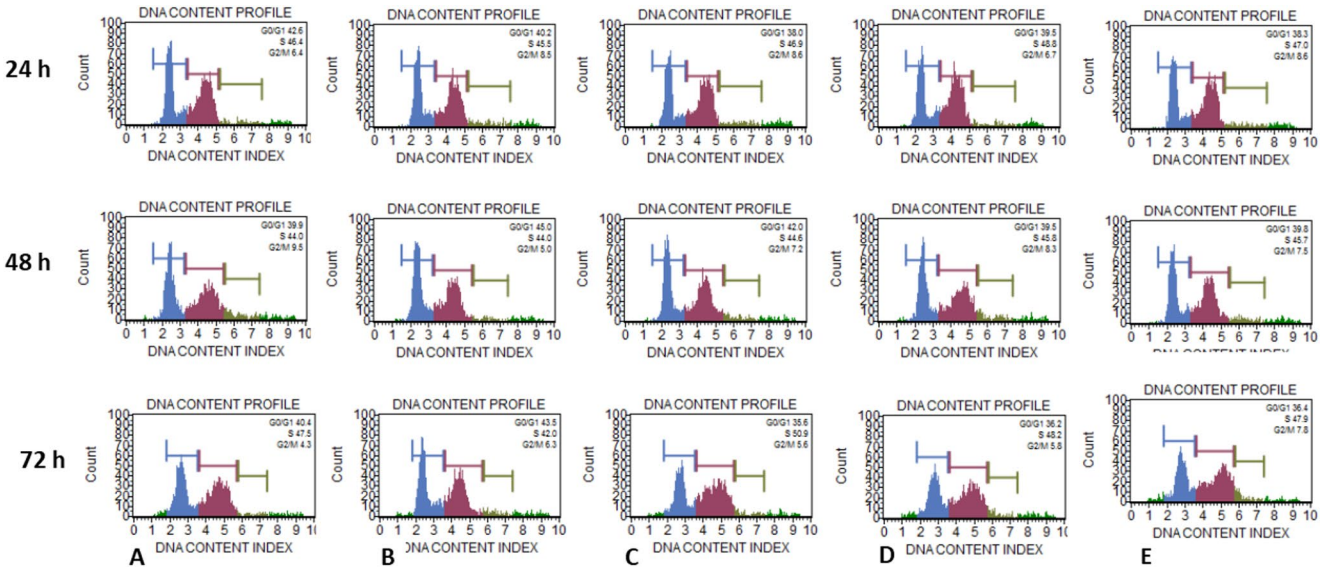

Fig. 3 Cell cycle assay: Data plots were obtained from the Muse Cell Analyzer after 24, 48, and 72 h of incubation. **A** EAT Control group, **B** EAT + 5 FU group, **C** EAT + 25 µg/ml DCE group, **D** EAT + 50 µg/ml DCE group, **E** EAT + 100 µg/ml DCE group

Table 4 EAT cell rate at the G0/G1, S, and G2/M phases (%) (24, 48, and 72 h incubation)

Hours /Groups	EAT Control (A)	EAT + 5-FU (B)	EAT + 25 µg/ml DCE (C)	EAT + 50 µg/ml DCE (D)	EAT + 100 µg/ml DCE (E)	*p*
24 h ($G_0/G_{1)}$	42,6(40,8–43,8)	43 (40,2–44,8)	41,2 (38–41,9)	40,9 (39,5–42,4)	37 (38,3–36,9)	0,095
48 h ($G_0/G_{1)}$	39,9 (39,6–42,4)	45 (39,7–47)	42 (36,4–43,2)	39,5 (39,4–40)	39,8 (39,1–41,3)	0,473
72 h ($G_0/G_{1)}$	40,4(39,1–41,3)[a]	43,5 (42,9–44)[b]	36,4 (35,6–37,5)[a]	36,2 (32,6–38,6)[a]	36,4 (36,1–39,1)[a]	0,029*
24 h (S)	45,8 (43,9–46,4)	44,4 (42,8–45,5)	46,2 (44,7–46,9)	47,3 (46–48,8)	47,1 (47–48,4)	0,05
48 h (S)	42,9 (4,8–44)	43,6 (43,2–44)	44,6 (42–48,1)	45,8 (45,4–47,4)	45,7 (43,6–45,9)	0,17
72 h (S)	46,4 (46–47,5)[ab]	41 (40,8–42)[a]	50 (47,6–50,9)[b]	48,2 (47–49,5)[ab]	47,9 (47,5–49,8)[ab]	0,03*
24 h (G_2/M)	6,9 (6,4–8,1)	7,3 (6,3–8,5)	7,7(7,1–8,6)	6,6 (6,5–6,7)	8,6 (8,6–9,6)	0,073
48 h (G_2/M)	9 (7,4–9,5)	5 (4–8,6)	8 (7,2–10,4)	7,9 (7,1–8,3)	7,5 (7,2–7,9)	0,475
72 h (G_2/M)	4,5 (4,3–7,1)	6,9 (6,3–36,3)	5,6 (5–6,2)	5,8 (5,4–8,8)	6,2 (5,6–7,8)	0,314

significant difference between the groups containing the same letter ([a, b,c]) ($p > 0,05$)

In vivo results

Immunohistochemistry results

TNF-α expression showed a significant increase in liver and kidney samples of the EAT Control group ($p < 0.05$). The 75 and 150 mg/kg DCE groups were similar to the Control group. In kidney tissue, the EAT + 5-FU, EAT + 75 mg/kg DCE, and EAT + 150 mg/kg DCE groups were similar, with a significant decrease compared to the EAT + Control group ($p < 0.05$). In liver tissue, the EAT + 5-FU and EAT + 75 mg/kg DCE groups were similar, with a significant decrease compared to the EAT + Control group ($p < 0.05$) (Figs. 4 and 5).

TAS-TOS results

The renal and hepatic TAS levels were statistically significantly decreased in the EAT + Control group compared to the control group ($p < 0.05$). However, in the EAT + 75 mg/kg DCE group and EAT + 150 mg/kg DCE group, TAS levels showed a statistically significant increase compared to the EAT + Control group ($p < 0.05$). Additionally, renal and hepatic TOS levels significantly increased in the EAT + Control group compared to the control group ($p < 0.05$). Conversely, in the EAT + 75 mg/kg DCE group and EAT + 150 mg/kg DCE group, TOS levels significantly decreased compared to the EAT + Control group ($p < 0.05$) (Table 5).

Comet analysis results

Comet assay findings in kidney and liver tissues demonstrated a marked increase in DNA damage in the EAT + Control group. In the kidney, the tail DNA percentage, tail length, and tail moment were measured as 92.5%,

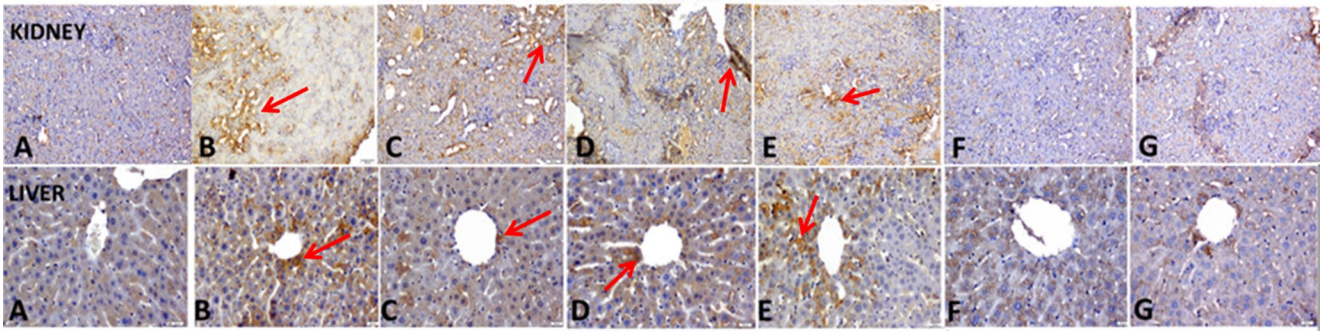

Fig. 4 Immunostaining images of TNFα protein in kidney and liver tissues of mice (**A**: Control group, **B**: EAT + Control group, **C**: EAT + 5-FU group, **D**: EAT + 75 mg/kg DCE group, **E**: EAT + 150 DCE group, F: 75 mg/kg DCE group, **G**: 150 mg/kg DCE group). Localizations of the protein in the tissue sections of the experimental groups (Focus 40X, bar 20 µm)

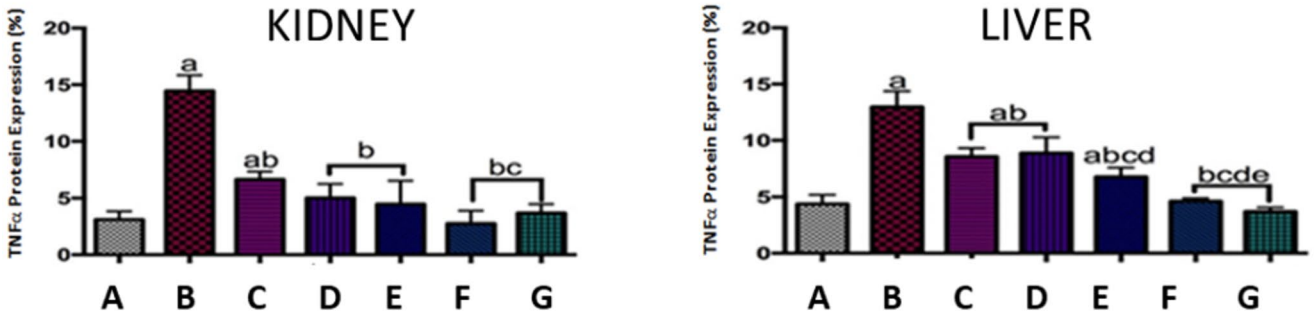

Fig. 5 The data shown in the histogram graph represents. TNF-α immunoreactivity intensity is expressed as mean ± SD. One-way ANOVA variance analysis and Tukey's multiple comparison test were applied (**A**: Control group, **B**: EAT + Control group, **C**: EAT + 5-FU group, **D**: EAT + 75 mg/kg DCE group, **E**: EAT + 150 DCE group, **F**: 75 mg/kg DCE group, **G**: 150 mg/kg DCE group) (a, differs from the control group; b, differs from the EAT + control group; c, differs from the EAT + 5-FU group; d, differs from the EAT + 75 mg/kg group; e, differs from the EAT + 150 mg/kg group, $p < 0.05$)

Table 5 TAS-TOS levels

TAS (mmol/L) TOS (µmol/L)/Groups	Control	EAT Control	EAT + 5-FU	EAT + 75 mg/kg DCE	EAT + 150 mg/kg DCE	75 mg/kg DCE	150 mg/kg DCE	*p*
Kidney TAS	$1,3 \pm 0,2^{a}$	$0,4 \pm 0,04^{b}$	$1,1 \pm 0,1^{a}$	$1,1 \pm 0,3^{a}$	$1,1 \pm 0,2^{a}$	$1,3 \pm 0,2^{a}$	$1,3 \pm 0,2^{a}$	0,0001*
Kidney TOS	$9,8 \pm 1,9^{a}$	$15,4 \pm 1,6^{b}$	$10 \pm 1,6^{a}$	$10,2 \pm 1,8^{a}$	$10,3 \pm 1,1^{a}$	$8,7 \pm 1,1^{a}$	$9,3 \pm 1,7^{a}$	0,0001*
Liver TAS	$2,4 \pm 0,2^{ac}$	$1,1 \pm 0,1^{b}$	$2,1 \pm 0,1^{ac}$	$2,1 \pm 0,1^{a}$	$2,1 \pm 0,3^{a}$	$2,5 \pm 0,1^{c}$	$2,4 \pm 0,1^{ac}$	0,0001*
Liver TOS	$10,8 \pm 1,1^{a}$	$14,4 \pm 2^{b}$	$11,2 \pm 0,8^{a}$	$11,3 \pm 1,2^{a}$	$11,3 \pm 1,2^{a}$	$9,4 \pm 1,2^{a}$	$9,6 \pm 1,3^{a}$	0,0001*

$p < 0,05$ was considered statistically significant. Data are expressed as mean ± SD (standard deviation). There is no statistically significant difference between the groups containing the same letter ($^{a, b,c}$) ($p > 0,05$)

61.8 µm, and 57.1, respectively. In the liver, these values were 105.3%, 50.9 µm, and 53.6, all showing statistically significant increases compared to the control group ($p < 0.0001$). In the EAT + 5-FU and DCE-treated groups, these parameters were significantly reduced. Notably, in the group treated with 150 mg/kg DCE, the kidney tail DNA percentage decreased to 34.8%, tail length to 15.1 µm, and tail moment to 5.2. Similarly, in the liver tissue, the 150 mg/kg DCE group showed substantial reductions in DNA damage (65.8% tail DNA, 17.6 µm tail length, and 11.6 tail moment). DNA damage levels in groups receiving DCE alone were significantly lower than in the EAT + Control group and were close to those of the healthy control group (Table 6; Fig. 6). These results indicate that DCE does not exhibit genotoxic effects and provides dose-dependent protection against EAT-induced DNA damage.

Discussion

Antioxidants exert protective effects against tumor formation by neutralizing oxidants through mechanisms such as scavenging free radicals, suppressing the formation of reactive species, repairing oxidative damage, and interrupting chain reactions (Tang et al. 2023). Phenolic and flavonoid compounds, abundantly found in plants, are among the

Table 6 The comet analysis results for kidney and liver tissues

Comet Analysis /Groups	Control	EAT Control	EAT + 5-FU	EAT + 75 mg/kg DCE	EAT + 150 mg/kg DCE	75 mg/kg DCE	150 mg/kg DCE	*p*
Kidney Tail DNA % ±SD	25,6 ± 0,1[a]	92,5 ± 2,1[b]	58,94 ± 9,3[c]	48,2 ± 2,7[d]	34,8 ± 6,2[e]	29,5 ± 23,3[a]	29,2 ± 3,2[a]	0,0001*
Kidney Tail Lenght ± SD	12,6 ± 1,2[a]	61,8 ± 2,2[b]	37,2 ± 19,2[c]	25,8 ± 1,1[d]	15,1 ± 2,2[e]	17,1 ± 9,2[a]	12,4 ± 7,2[a]	0,0001*
Kidney Tail Moment ± SD	3,2 ± 0,1[a]	57,1 ± 0,1[b]	21,9 ± 1,8[c]	12,4 ± 0,1[d]	5,2 ± 0,1[e]	5,±2,2[a]	3,6 ± 0,2[a]	0,0001*
Liver Tail DNA % ±SD	26,5 ± 2,3[a]	105,3 ± 8,4[b]	85,3 ± 7,3[c]	72,3 ± 5,2[d]	65,8 ± 2,8[e]	45,6 ± 13,2[f]	51,7 ± 2,4[f]	0,0001*
Liver Tail Lenght ± SD	4,6 ± 1,3[a]	50,9 ± 7,2[b]	11,1 ± 1,1[c]	32,1 ± 3,2[d]	17,6 ± 6,1[e]	22,5 ± 7[f]	24,3 ± 3,2[f]	0,0001*
Liver Tail Moment ± SD	1,2 ± 0,3[a]	53,6 ± 0,6[b]	9,4 ± 0,7[c]	23,2 ± 0,2[d]	11,6 ± 0,2[e]	10,3 ± 0,9[f]	12,5 ± 0,1[f]	0,0001*

$p < 0,05$ was considered statistically significant. Data are expressed as mean ± SD (standard deviation). There is no statistically significant difference between the groups containing the same letter ([a, b,c]) ($p > 0,05$)

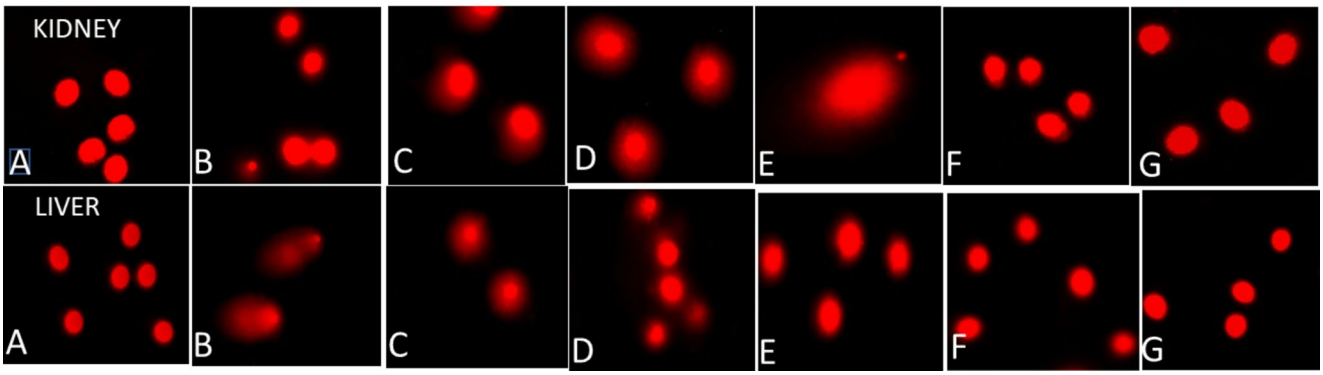

Fig. 6 Typical comet images of kidney and liver tissue cells (**A**: Control group, **B**: EAT + Control group, **C**: EAT + 5-FU group, **D**: EAT + 75 mg/kg DCE act group, **E**: EAT + 150 DCE group, F: 75 mg/kg DCE group, G: 150 mg/kg DCE group)

primary contributors to antioxidant capacity due to their strong redox properties (Zivari-Ghader et al. 2024). Various studies examining the phenolic and flavonoid profiles of GC extracts obtained from seeds or leaves have reported significant variability in their content, which is generally attributed to the extraction method and the plant material used. For example, total phenolic content (TPC) in garden cress seed extracts obtained by two methods was determined as 88.08 and 126.24 $mg_{GAE}/g_{extract}$, respectively, indicating a method-dependent yield (El Maati et al. 2016). Other studies have reported TPC values ranging from 184.14 to 374 $mg_{GAE}/g_{extract}$, while total flavonoid content (TFC) has been reported between 12.63 and 51 $mg_{CAE}/g_{extract}$ (Selek et al. 2018; Abdel-Aty et al. 2019). Additionally, in a study comparing different parts of the plant, ethanolic leaf extracts (10 mg/mL) were found to contain higher levels of reduced glutathione than seeds, suggesting that leaf tissue may have greater radical scavenging capacity (Malar et al. 2014). Similarly, the amount of BITC in Lepidium sativum extracts varies depending on the extraction method. Previous studies have reported BITC levels ranging from 2.04 to 3.82 µg/g, depending on the solvent and method employed, with the highest yield obtained from sprouts extracted by supercritical fluid extraction (SFE) (Rafińska et al., 2019). Moreover, methods such as Clevenger-type hydrodistillation, modified simple distillation, and simultaneous distillation-extraction (SDE) have been shown to influence the composition of volatile compounds, with the SDE method particularly enhancing BITC recovery and biological activity (Getahun et al., 2020). In our study, the total phenolic content was measured as 159.62 ± 2.07 $mg_{GAE}/g_{extract}$, total flavonoid content as 97.28 ± 4.35 $mg_{CAE}/g_{extract}$, and BITC content in a DCE prepared at 1 mg/mL concentration was determined to be 1.787 mg/L. The TPC and TFC values align with those reported in the literature; however, differences in measurement units complicate direct comparison of BITC content. However, it should be considered that factors such as climatic conditions, agricultural practices, harvesting and storage methods, the plant part used, and extraction protocols can significantly affect phytochemical content.

In the apoptotic cascade, a decrease in mitochondrial membrane potential leads to the opening of mitochondrial transition pores, releasing cytochrome-c and apoptosis-inducing factor into the cytoplasm, which initiates apoptosis (Lu et al. 2018). A study on the methanolic extract of garden cress demonstrated concentration-dependent cytotoxicity in colon and endometrial cancer cells, with significantly increased apoptosis and genotoxic effects observed particularly at 200 µg/mL after 48 h of incubation (Selek et al. 2018). Similarly, curcumin-loaded selenium nanoparticles showed antitumor activity in HCT116 cell lines and EAT solid tumors by promoting cytochrome c release from

mitochondria, which led to apoptosis and a reduction in tumor burden in vivo (Kumari et al. 2017). Additionally, a pyrrole-based compound from Tinospora cordifolia was reported to induce reactive oxygen species (ROS) generation and mitochondria-mediated apoptosis by restoring p53 activity in breast cancer cells in vitro, while inhibiting tumor proliferation through EAT cell death in vivo (Rashmi et al. 2019). Furthermore, an in vitro study on benzyl isothiocyanate (BITC) revealed that it triggers apoptosis in human lung cancer cells via mitochondrial and caspase-dependent pathways (Huang et al. 2018). In line with these findings, our in vitro results showed a statistically significant change in mitochondrial membrane potential after treating cells with 100 µg/mL of DCE for 24 h, suggesting that apoptosis may be induced through mitochondrial pathways. These results are consistent with the literature demonstrating that different plant extracts and compounds induce mitochondria-mediated apoptosis, showing antitumor effects in experimental models such as the EAT tumor or various cell lines treated with DCE. However, comprehensive molecular studies are needed to understand these mechanisms better.

Initiator caspases activate caspase-3 and caspase-7 in response to cell death-inducing cytokines, serving as key markers of apoptosis (Ai et al. 2024). Studies on garden cress extract have shown variable effects: in breast cancer cells, caspase-3 levels remained unchanged at apoptotic doses, suggesting alternative apoptosis pathways, whereas in leukemia cells, caspase-3 activation peaked after 24 h of incubation (Mahassni & Al-Reemi, 2013; Basaiyye et al. 2019). In our in vitro study, the highest apoptotic cell death mediated by caspase pathways was observed at a 50 µg/mL dose of LS after 48 h, indicating that apoptosis may be induced via caspase-3/7 activation. These findings are consistent with previous reports highlighting the role of caspase activation in garden cress-induced apoptosis. However, variations among different cancer cell types suggest the involvement of multiple apoptotic pathways.

Checkpoints during cell division are critical for maintaining genetic integrity by allowing only cells with correctly replicated DNA to proceed through the cell cycle. In cancer, these checkpoints are often disrupted, leading to uncontrolled cell proliferation and tumor growth (Jamasbi et al. 2022). Various studies have shown that different plant extracts can exert antitumor effects by arresting the cell cycle at specific phases in cancer cells. Notably, acetone extract of garden cress seeds (LSSAExt) and biogenic silver nanoparticles (AgNPs), either alone or in combination, inhibited the division of colon cancer cells (Ibrahim et al. 2021). Cell cycle analysis was performed in several extract studies using the EAT model. In one study where pea lectin was administered intraperitoneally, 52% of cells were in the G0/G1 phase, 0% in the S phase, and 48% in the G2/M phase (Kabir et al. 2013). Pecan extract increased the number of tumor cells in the G1 phase while decreasing them in the G2/M phase, possibly by activating key proteins such as cyclin A, cyclin B, and CDK2 (Hilbig et al. 2018). Trichosanthes dioica seed extract inhibited cell proliferation by arresting the cell cycle in the G0/G1 phase (Islam et al. 2021). Our in vitro study demonstrated that 5-FU induced cell cycle arrest in the G0/G1 phase, while DCE at 25 µg/mL triggered cell accumulation in the S phase. While 5-FU blocks the cell cycle at an early phase, DCE may slow progression primarily by interfering during DNA replication. Thus, both compounds may exert antitumor effects by targeting different cell cycle checkpoints. At 48 and 72 h, no significant effects were observed compared to the earlier time points. This may be explained by the fact that the cytotoxic or biological activity of the DCE peaks at earlier time points, after which cellular adaptive mechanisms or degradation of the extract reduce its efficacy over time. Additionally, metabolic processes and compound stability under experimental conditions may influence this time-dependent decline in activity. These factors highlight potential limitations of the extract's sustained effect and suggest that further kinetic and mechanistic studies are needed to fully understand its temporal dynamics.

Tumor necrosis factor alpha (TNF-α) is a cytokine with dual roles, functioning as pro-inflammatory and proapoptotic factors (Ai et al. 2024). According to our in vivo findings elevated TNF-α levels in the EAT control group likely indicate increased tumor-associated inflammation. Conversely, the decrease in TNF-α levels following DCE treatment suggests an antiinflammatory effect and suppression of tumor progression. However, considering the proapoptotic role of TNF-α, this reduction may reflect a complex regulatory response. In some contexts, decreased TNF-α could lead to reduced apoptosis. Therefore, changes in TNF-α levels are context-dependent and multifactorial. Various studies support the antiinflammatory potential of garden cress extract. For instance, TNF-α levels were significantly reduced in human keratinocyte cells treated with garden cress leaf extract (Türkoğlu et al., 2018). Additionally, garden cress seed oil regulated TNF-α levels in Wistar rats with induced ulcerative colitis, and another study showed that garden cress seeds reduced TNF-α levels in the plasma of E. coli-stimulated mice (Reddy et al. 2014; Ahmad et al. 2018). Marine-derived Penicillium purpurogenum reduced solid EAT tumor size and serum TNF-α levels (Teles et al. 2020). On the other hand, a piperine analog extract was suggested to stimulate the immune response by increasing TNF-α levels in EAT peritoneal ascitic fluid (Ferreira et al. 2020). Similarly, quercetin decreased TNF-α concentrations in the spinal cord and paw skin in solid EAT tumor models (Calixto-Campos et al. 2015). Our findings align with these

results; DCE reduced TNF-α expression in EAT-bearing subjects' kidney and liver tissues, indicating suppression of the immune response in these organs. Notably, TNF-α levels remained unchanged in non-tumor groups, suggesting that DCE does not cause immunomodulatory effects in healthy tissues. Overall, our results are consistent with the literature; however, further research is needed to understand better the role of TNF-α in the antitumor effects of DCE. Determining whether this modulation primarily reflects antiinflammatory activity or involves additional mechanisms, including apoptosis regulation, is important.

In solid EAT tumors treated with avenanthramide, antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) were reported to increase. At the same time, the oxidative stress marker malondialdehyde (MDA) decreased in the treated group (Aldubayan et al. 2019). Similarly, cranberry and fenugreek extracts increased total antioxidant status (TAS) and decreased total oxidant status (TOS) in tumor tissue as well as in the lungs, brain, kidneys, liver, and testes (Nisari et al., 2017; Yılmaz et al., 2020). Our in vivo findings showed high oxidative stress and low antioxidant capacity in tumor tissues of the control group. In contrast, the increased TAS levels observed in the groups treated with DCE may indicate a possible antioxidant and antitumor effect of this extract.

Tumors may disrupt homeostasis in organs or tissues and lead to DNA damage. The comet assay quantitatively measures tissue DNA damage (Kiraz et al. 2016; Lu et al. 2017). In a study examining the antitumor effect of a nano-capsule without lipoic acid in solid EAT tumors, a 3- to 10-fold increase in DNA damage parameters was observed in tumor tissue, indicating suppression of Ehrlich solid tumor growth (Rageh and El-Gebaly 2019). Similarly, silver nanoparticles (AgNPs) increased comet assay parameters 2- to 3-fold in tumor tissue of EAT-bearing mice (Rageh et al. 2018). Garden cress seed extract protected against glutamate-induced excitotoxicity by reducing comet parameters and improving the viability of retinal ganglion cells (Al-Dbass et al. 2021). Our in vivo results indicate that, DCE did not cause DNA damage in kidney tissue of healthy mice, whereas a significant increase in comet parameters was observed in liver tissue. However, EAT increased DNA damage in both kidney and liver tissues. Both DCE and 5-FU effectively reduced DNA damage induced by EAT. Interestingly, kidney tissue responded more than liver tissue, with tail parameters closer to healthy controls. This may indicate organ-specific sensitivity or metabolic differences. Overall, the findings suggest that DCE may play a role in reducing genotoxic stress caused by EAT; however, more comprehensive studies are needed to elucidate the exact mechanisms involved.

The present study evaluated the in vitro and in vivo anticancer effects of the DCE. However in vitro analyses were not performed to determine its direct cytotoxic effects on cancer cells (such as MTT or SRB assays). The study did not include complementary in vitro tests such as Annexin V staining or DNA fragmentation analysis. This represents a significant limitation that should be addressed in future research to define the mechanism of cell death more clearly. In the study, DCE administration showed biological effects similar to those of 5-FU in some parameters. However, 5-FU is a clinically established anticancer agent. Therefore, direct comparisons between DCE and 5-FU should be made cautiously. Although DCE induced significant biological responses at specific doses, these findings require validation through broader experimental and molecular analyses. Future studies involving combination models of DCE and 5-FU may clarify possible synergistic or complementary interactions between the two agents. Furthermore, the pharmacokinetic behavior and bioavailability of DCE in humans remain unclear, which may limit its therapeutic applicability. Detailed safety evaluations, including toxicological effects, have not yet been conducted. Additionally, the study was performed with a limited number of animals of a single sex, which restricts the generalizability of the results. The absence of pharmacodynamic data also limits understanding of the compound's mechanism of action. These limitations highlight the need for more comprehensive and multifaceted studies before the findings can be clinically relevant.

In conclusion, our findings suggest that DCE may induce apoptosis in EAT cells in vitro through caspase-3/7 activation and could contribute to mitochondrial depolarization and the regulation of cell cycle checkpoints. In the in vivo study, lower TNF-α levels, increased TAS, decreased TOS values, and reduced genotoxic damage were observed in treated animals' kidney and liver tissues, indicating that DCE may exhibit antioxidant and antitumor properties under experimental conditions. These findings provide a preliminary basis for further experimental studies to explore the likely biological effects of DCE in oncological research. To better explore the translational potential of these findings, additional studies using tumor models that more closely resemble human cancer biology (such as MCF-7, BT-474, A549, PC-3) are needed. Moreover, evaluating molecular markers such as Bax, Bcl-2, and Annexin V is important to understand better the mechanisms underlying DCE's observed effects. These comprehensive approaches may contribute to a deeper understanding of DCE's therapeutic prospects and safety profile in preclinical cancer research.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval The applications performed on experimental animals were evaluated in terms of animal rights and welfare, and their suitability in terms of animal experiments ethics was approved by the Erciyes University, Experimental Animals Local Ethics Committee, decision number 19/174 dated 09.10.2019.

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