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RESEARCH ARTICLE

Antitumor effects of geraniol on oral cancer

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Abstract

***Background*:** Geraniol has been shown to possess therapeutic or preventive effects against various types of human cancers but not affect normal physiology through regulating cell cycle and apoptosis. However, the biological effects of geraniol on oral squamous cell carcinoma (OSCC) cells are unknown.
***Methods*:** OSCC cell proliferation was measured by cell-counting kit-8 and colony formation assays. Cell migration was assessed by wound-healing assay. Apoptosis of OSCC cells was detected by TUNEL staining analysis. UM1 xenograft mouse model was used for determining the antitumor effects of geraniol in vivo.
***Results*:** Geraniol treatment significantly suppressed OSCC cell proliferation and migration in vitro, and tumor growth in vivo in a time- and dose-dependent manner. Further investigation showed that geraniol treatment effectively induced OSCC apoptosis and blocked Phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) signaling activation concurrently. We also found that geraniol administration at the dose of 100 and 250 mg/kg did not affect the body weight on tumor-bearing mice, demonstrating the safety of geraniol.
***Conclusion*:** Geraniol may serve as a promising anticancer drug for the treatment of oral cancer.

Keywords: *geraniol*; *oral cancer*; *cell proliferation*; *cell migration*; *tumor growth*

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Excessive alcohol consumption is also accounted for oral cancer occurrence in both east and west world (1, 2). Oropharynx cancers have linked to human papillomavirus infection and lip cancer to excessive sunlight exposure (3). Oral cancer patients diagnosed at stages 1 or 2 are generally treated with surgery, and with a combination of surgery and adjuvant therapy (e.g. chemotherapy or chemoradiation) for oral cancer at stages 3 and 4 (4, 5). Despite the great advance in cancer therapy development, the overall 5-year survival rate does not change significantly. Studies have revealed that recurrence happens in 35% of oral cancer patients (6, 7). Therefore, developing a new strategy for oral cancer treatment or prevention is extremely important.

It is known that nature food (e.g. vegetables and fruits) may exert antitumor effects on various human cancers. Several flavonoids, such as galangin, apigenin, luteolin, and catechins, have been demonstrated to possess protective effects on healthy normal cells while strong anticancer activities to cancer cells (8–10). Therefore, it is reasonable to explore nature chemicals as a potential candidate for cancer treatment. Geraniol is commonly found in the essential oils from many aromatic plants, such as Cymbopogon martinii var motia, Pelargonium graveolens, Cymbopogon nardus L., and Cymbopogon jwarancusa (11, 12). Decades of studies have revealed that geraniol possesses various pharmacological effects, such as antioxidant, anti-inflammatory, and neuroprotective activities (11–13). Geraniol also gained increasing attention for its potential role against a broad spectrum of human cancers, including lung, prostate, colon, breast, ovarian, and pancreatic cancers (14–16). Madankumar et al. showed that administration of geraniol was able to inhibit oral cancer initiation and progression (17). However, the effect of geraniol on oral cancer is less well studied, especially for its detailed mechanisms.

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Materials and methods

Cell culture

UM1 and SCC9, two types of oral squamous cell carcinoma (OSCC) cell lines, were purchased from ATCC (Manassas, VA). UM1 and SCC9 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Logan, UT) supplemented with fetal bovine serum (10%, Hyclone).

Cell proliferation assay

Cell Counting Kit-8 (CCK-8, Sigma, St. Louis, MO) was employed to assess cell proliferation. UM1 or SCC9 cells were seeded at a density of 1,000/well with or without vehicle (dimethyl sulfoxide, DMSO) or geraniol (25, 50, and 100 μM) and were cultured for 24, 48, and 72 h. After that, 10 μL of CCK8 solution was added into each well, following incubation for 4 h. The absorbance at 450 nm was recorded using a microplate reader.

Colony formation assay

UM1 or SCC9 cells were seeded at a density of 3,000/well with vehicle (DMSO) or geraniol (25, 50, and 100 μM). Cells were cultured in a incubate for 14 days. Colonies were fixed with 100% methanol and stained with crystal violet for 5 mins. The plate was washed with phosphate-buffered saline and dried overnight. Colonies' pictures were taken by an inverted microscope.

Wound healing assay

UM1 or SCC9 cells were seeded in a 6-well plate until reaching 100% confluence. A 100 μm wide vertical line was created crossing each well using a pipette tip. Cells were cultured with vehicle (DMSO) or geraniol (25 and 100 μM) for 48 h. The images were taken by an inverted microscope, and the gap closure was determined.

TUNEL staining assay

The apoptotic cells were detected by TUNEL Assay Kit (Abcam, Cambridge, MA). Briefly, UM1 or SCC9 cells were seeded above a coverslip and were cultured with vehicle (DMSO) or geraniol (25 and 100 μM) for 48 h. Coverslip was removed from the plate, washed, and covered with the DNA labeling solution. After incubation for 1 h, coverslip was washed and covered with anti-BrdU-Red antibody solution. Thirty minutes later, coverslip was incubated in the staining buffer in the dark for 30 min. After sealing the coverslip with rubber cement, the apoptotic cells on the coverslip can be examined under a microscope.

Western blot analysis

Ice-cold radioimmunoprecipitation buffer with a protease inhibitor was used to extract the protein. After separating by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, the protein was transferred to a polyvinylidene fluoride membrane. After incubation with corresponding primary and second antibodies, the chemo-luminescent signal was examined using the electrochemiluminescence (ECL) system (Thermo, Waltham, CA).

Animal experiment

Balb/c nude mice were used. A total of 1×10^6 UM1 cells were injected into the right flank of each nude mouse. The tumor-bearing mice were received intragastric administration of corn oil (control), geraniol at 100 mg/kg, and geraniol at 250 mg/kg. The body weight and tumor size were monitored and recorded every 3 days. Tumor tissues were collected and weighted after 28-day post-tumor implantation. This study was approved by Matrouh University.

Statistics

Data were presented as mean ± standard deviation (SD) and analyzed by two-way ANOVA or one-way ANOVA with a Bonferroni's post hoc test, or one-way ANOVA with a Tukey's post hoc test.

Results

Geraniol inhibits cell proliferation of OSCC cells

As shown in Fig. 1A and B, geraniol dose-dependently inhibited the cell proliferation of UM1 and SCC9 with the strongest inhibition effect at 100 μM. Similarly, UM1 and SCC9 cells were treated with vehicle or geraniol and subjected to colony formation assay. The results also revealed that geraniol potently suppressed colony formation of UM1 and SCC9 in a dose-dependent manner (Fig. 1C and D). These results suggested that geraniol is a potent cell growth inhibition reagent for OSCC cells.

Geraniol suppresses cell proliferation of OSCC cells

Next, we aimed to explore the effect of geraniol on the cell migration. UM1 and SCC9 cells were treated with vehicle or two different doses of geraniol (25 and 100 μM) and were subjected to wound healing assay. After 48 h of culture, the gap closure in vehicle-treated UM1 and SCC9 was significantly reduced in geraniol-treated UM1 and SCC9 (Fig. 2A–B). These results showed that geraniol is able to suppress cell migration of OSCC cells.

Geraniol induces apoptosis of OSCC cells

Previous studies have demonstrated that geraniol treatment can induce proapoptotic protein expression, causing apoptosis of cancer cells (18). To confirm this finding, we treated UM1 and SCC9 cells with vehicle or two different doses of geraniol (25 and 100 μM). The apoptotic cells were analyzed by tunnel staining. The results showed that geraniol treatment induced a significant number of apoptotic cells of UM1 and SCC9 (Fig. 3A–B). Consistent with others' findings, we also found that geraniol treatment

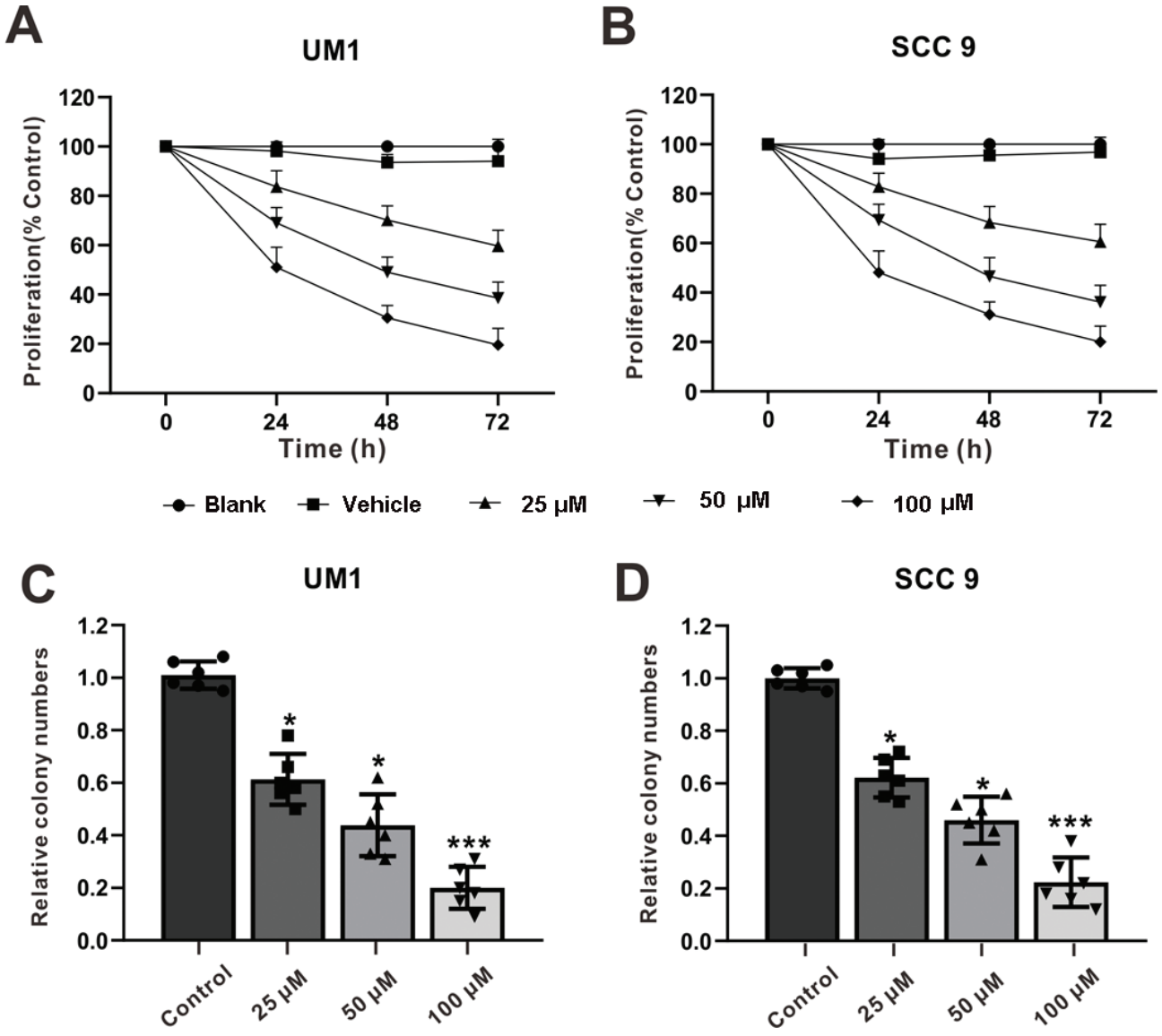

Fig. 1. Geraniol inhibits proliferation and colony formation of UM1 and SCC 9 cells in a time- and dose-dependent manner. (A and B) Cells were treated with vehicle or different concentrations of geraniol as indicated. Cell viability was determined by Cell Counting Kit-8 (CCK-8) assay at 24, 48, and 72 h following treatment. (C–D) Colony formation assay was performed to evaluate the colony formation ability of cells with vehicle or different concentrations of geraniol as indicated. $*P < 0.05$ and $***P < 0.001$, compared with the control group.

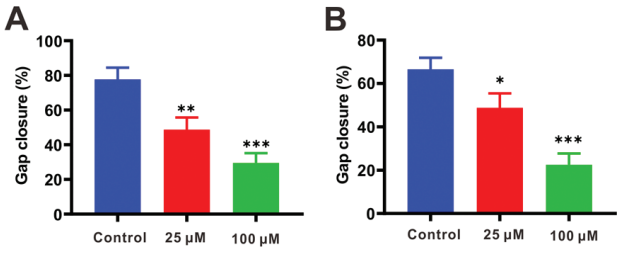

Fig. 2. Geraniol inhibits migration of UM1 and SCC 9 cells in a dose-dependent manner. (A–B) Wound healing assay was carried out to measure the migratory ability of indicated cells. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$, compared with the control group.

enhanced the expression of Bax, a proapoptotic protein, and reduced the expression of Bcl-2, an anti-apoptotic protein, in UM1 and SCC9 cells (Fig. 3C–D).

Geraniol inhibits PI3K/AKT signaling activation in OSCC cells

PI3K/AKT signaling plays an important role in promoting cell proliferation and migration. Therefore, we sought to study whether PI3K/AKT signaling activation was impeded under geraniol treatment. UM1 and SCC9 cells were exposed to geraniol at 0, 25, and 100 μM for 48 h. As expected, the results in Fig. 4A–D manifested that geraniol treatment substantially reduced t p-PI3K and p-AKT expression, but not total AKT.

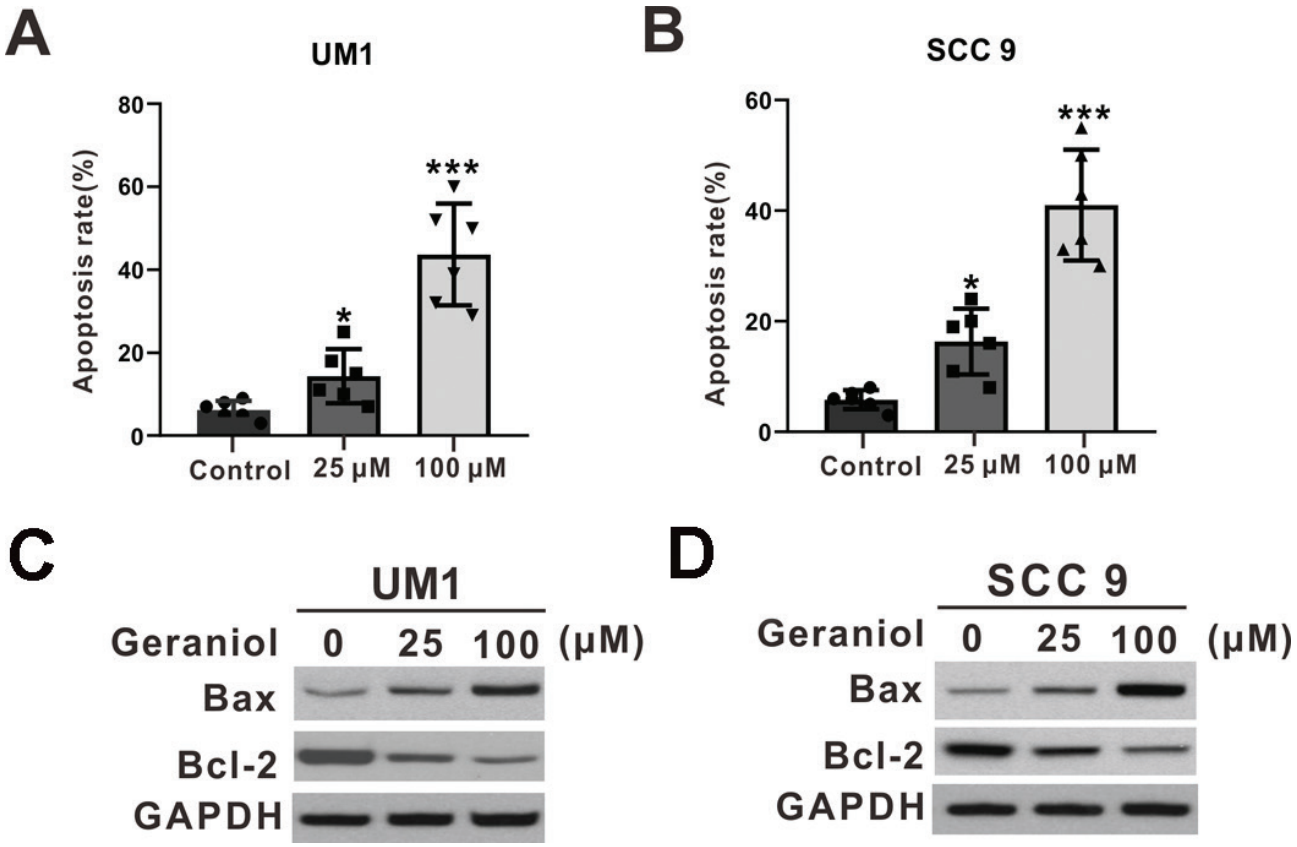

Fig. 3. Geraniol promotes apoptosis of UM1 and SCC 9 cells in a dose-dependent manner. (A–B) The apoptosis rate of indicated cells treated with or without geraniol at indicated concentrations was measured by TUNEL assay (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay). (C–D) The proteins associated with apoptosis were examined by Western blot. $*P < 0.05$ and $***P < 0.001$, compared with the control group.

Geraniol reduces tumor growth of OSCC

A previous study showed a markedly pancreatic tumor growth inhibition effect with geraniol feeding at 20 mg/g on Syrian golden hamsters (17). We hypothesized that administration of geraniol might also suppress OSCC tumor growth in vivo. The UM1 tumor-bearing mice were fed with a standard diet supplemented with geraniol at 0, 100, and 250 mg/kg. Tumor volume was measured at 3-day intervals. The results in Fig. 5A–C demonstrated that administration of geraniol dose-dependently inhibited tumor growth of UM1 in nude mice as demonstrated by reduced tumor volume and tumor weight in geraniol treatment groups. Notably, the body weight curve was comparable among all three groups, suggesting geraniol treatment at 100 and 250 mg/kg had no noticeable pharmacological toxicity.

Discussion

Here, we first demonstrated that geraniol inhibited cell proliferation and migration of UM1 and SCC9 cells in a time- and dose-dependent manner in vitro, and tumor growth of UM1 in vivo. We also found that geraniol treatment potently induced apoptosis of OSCC cells, suggesting that geraniol suppressed OSCC cell proliferation and migration through promoting apoptosis. Similar results have been reported in multiple human cancers with different mechanisms. For example, Duncan et al. reported that geraniol treatment causes G1 phase cell cycle arrest and inhibits cell growth of MCF-7 breast cancer cells (19). Carnesecchi et al. also showed that geraniol suppresses cell proliferation of Caco-2 colorectal adenocarcinoma cells by inducing S phase cell cycle arrest (16). Similarly, it was found that geraniol exerts an antiproliferative effect against human pancreatic cancer cells (20). Interestingly, Galle et al. reported that geraniol inhibits cell proliferation and tumor growth in vivo of A549 lung cancer cells (21). Correspondingly, geraniol-induced cell growth suppression and apoptosis have been observed in HepG2 liver cancer cells (22). Collectively, these studies suggest that geraniol inhibits various human cancer cell growth, but the mechanisms (cell cycle arrest or apoptosis) are tumor type-dependent.

We also revealed that the inhibition effects against OSCC cell proliferation and migration were around

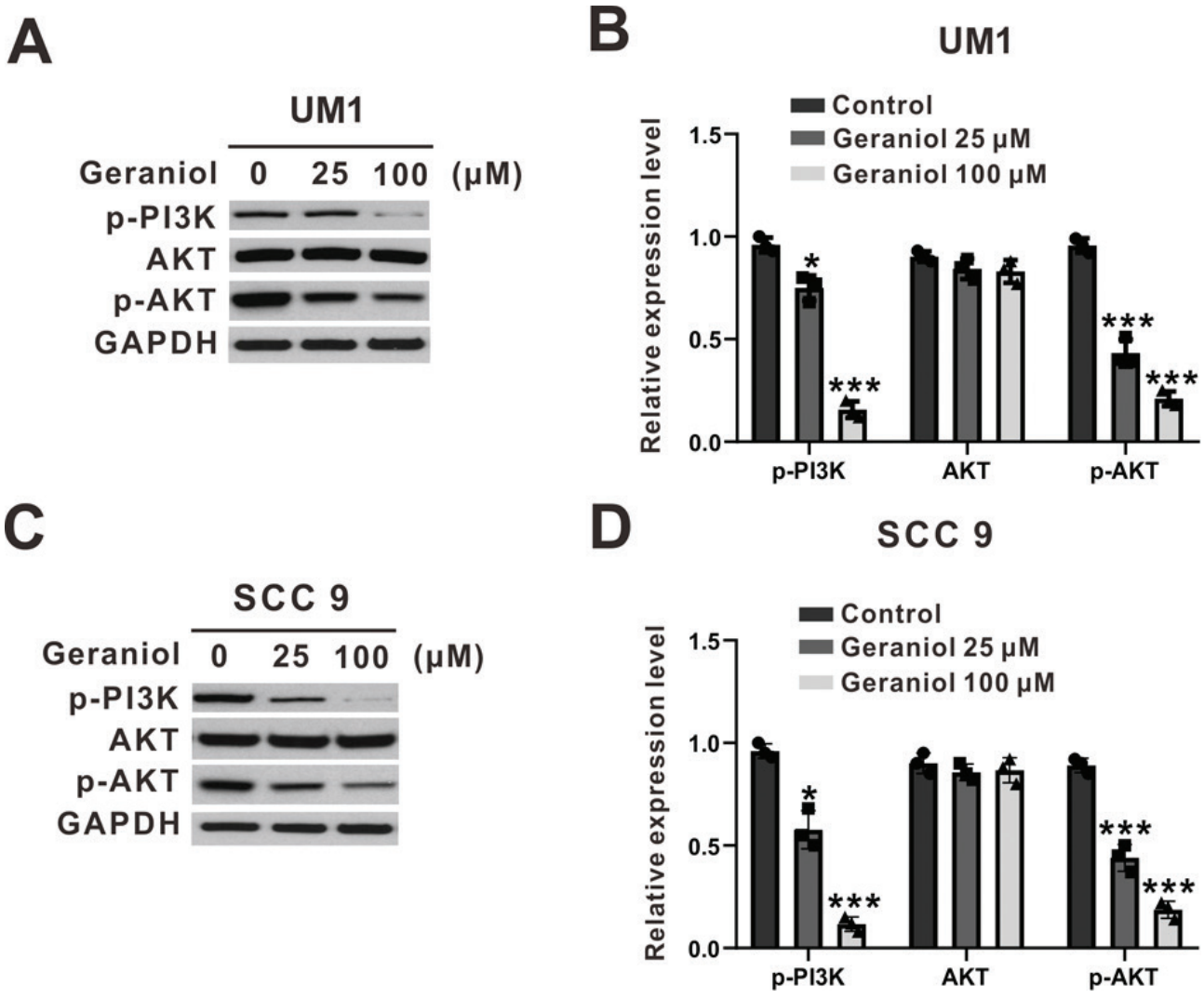

Fig. 4. Geraniol inhibited the PI3K/AKT signaling pathway. Cells were seeded and treated geraniol for 48 h. (A–D) The levels of PI3K, p-AKT, and AKT were determined by Western blot. The bar chart showed the quantitative results. Data are presented as means ± standard deviation (SD) for three independent experiments. *$P < 0.05$ and ***$P < 0.001$, compared to the control group.

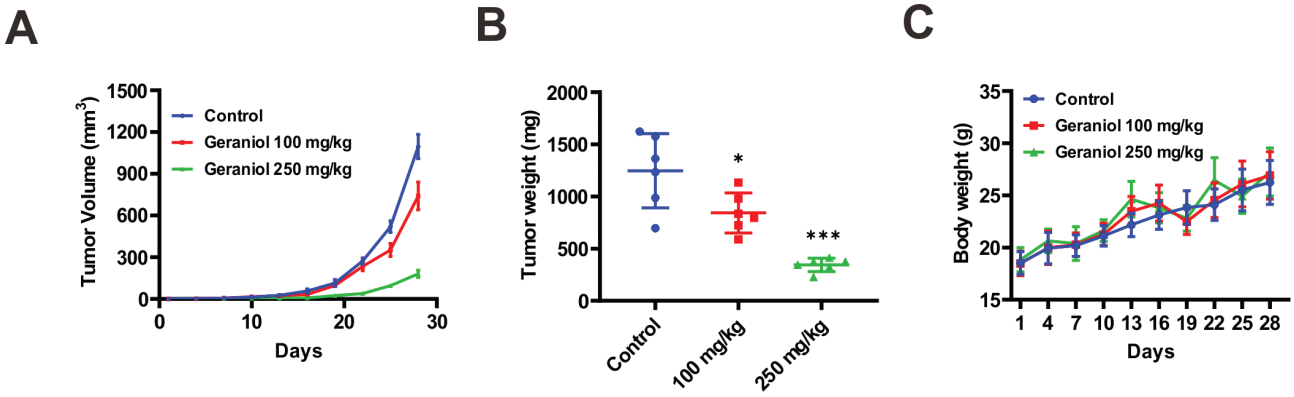

Fig. 5. The anticancer effect of geraniol in nude mice inoculated with UM1 cells. (A) The tumor volume was measured every 3 days. (B) The tumor weight was measured at the end of the experiment. (C) The body weight of mice was measured every 3 days. *$P < 0.05$ and ***$P < 0.001$, compared with the control group.

40–50% at low-dose geraniol (25 μM) and about 80% at high-dose geraniol (100 μM). We noticed that the effective doses of geraniol vary among different tumor types. For instance, the anti-proliferation effective doses of geraniol for MCF-7 range from 300 to 700 μM (19), for PC-3 range from 250 to 1,000 μM (23), for MIA PaCa-2 range from 100 to 500 μM (20), and for HepG2 range from 100 to 400 μM (22). We found that even the 'high-dose' (100 μM) used in our study is recognized as a shallow dose compared to these studies. Thus, it is intriguing to find that OSCC cells are susceptible to geraniol treatment. Furthermore, we demonstrated that based on tumor weight results, 50 and 80% tumor growth inhibition effects were achieved by oral administration of geraniol at doses of 100 mg/kg and 250 mg/kg, respectively. The tumor growth suppression effective dose of geraniol for A549 xenograft ranges from 4 to 12 g/kg (21); for PC-3 xenograft, it is 20 g/kg (23); for Morris 7777 hepatomas xenograft, it is 4 g/kg (24); and for B16 xenograft, it ranges from 1 to 10 g/kg (24, 25). Again, we proved that the antitumor effective doses of geraniol for oral cancer were significantly low compared to other tumor types of xenografts. Interestingly, similar results were reported by other groups. They showed that geraniol at 200 and 250–500 mg/kg exerts strong tumor inhibition effects on oral cancer (17, 26).

Finally, we demonstrated that geraniol inhibited cell growth and migration by blocking PI3K/AKT signaling activation. These results are also consistent with others' publications. Younis et al. reported that oral administration of geraniol mitigates myocardial infarction via suppressing PI3K/Akt/mTOR pathway, leading to inhibition of autophagy (27). Wu et al. showed that geraniol treatment suppresses PI3K/AKT and mitogen-activated protein kinase (MAPK) signaling activation in mice with osteoarthritis (28). We are the first to report that geraniol treatment is able to inhibit PI3K/AKT signaling activation in OSCC cells. However, the downstream targets of geraniol-mediated PI3K/AKT signaling pathway need further investigation.

In the current study, it is demonstrated that geraniol treatment suppresses PI3K/AKT signaling activation and induce apoptosis, resulting in strong inhibition effects on OSCC cell proliferation and migration in vitro and tumor growth in vivo. Geraniol may be a promising candidate for the treatment of oral cancer.

Conflicts of interest and funding

The authors declare that they have no competing interests. This work was funded by Research Funding in Matrouh University (2019.j62).

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