

## Original Research Article

# Elemene inhibits the growth and promotes apoptosis of bladder cancer cells through PTEN-Akt signaling pathway

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### Abstract

**Purpose:** To investigate the influence of elemene on growth and apoptotic changes in bladder cancer cells, and the involvement of the phosphatase and tensin homologous protein (PTEN)-serine/threonine kinase (Akt) signal pathway in the process.

**Methods:** Human bladder cell line SV-HUC-1 and human bladder cancer cell line T24 were randomly assigned to blank control group and elemene groups. Protein expression levels of apoptosis-related factors [Bcl-2, Bax, and caspase-3] and PTEN, Akt, and p-Akt in both cells were determined using western blot assay.

**Results:** In T24 cells, the protein level of Bcl-2 was significantly higher in elemene group than in blank control, while the protein expression levels of Bax and caspase-3 in elemene group were significantly lower than the corresponding levels in blank control group ( $p < 0.05$ ). The protein expression of PTEN level in T24 cells was significantly higher in elemene group than in blank control group, while protein expression level of p-Akt was significantly lower in elemene group than in blank control ( $p < 0.05$ ). The Akt protein expression was comparable in elemene and blank control groups.

**Conclusion:** Elemene inhibits the growth and enhances apoptosis of bladder cancer cells through a mechanism involving up-regulation of the expression of PTEN and suppression of the expression of p-Akt.

**Keywords:** Elemene, PTEN, Akt, bladder cancer

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## INTRODUCTION

Bladder cancer is derived from epithelial tissue, and the major pre-disposing factors are smoking and bladder infection [1]. Extant literature shows a high population of smokers in China. Moreover, the living standard of people in China is constantly improving. The

risk of bladder cancer is also increasing, a situation which poses serious threat to the lives and health of the general population [2]. At present, transurethral resection of bladder cancer is used for treating bladder cancer, with supplementation using chemotherapy drugs. The best drug recognized by clinicians at home and abroad for preventing cancer recurrence is

BCG. However, this drug causes many adverse reactions which limit its application [3]. Thus, it is important to discover more potent and safer chemotherapeutic medications to prevent bladder cancer recurrence.

Elemene is extracted from ginger plants. It is a new national class of anti-tumor drugs, with  $\beta$ -elemene as its main bioactive component.  $\beta$ -Elemene exerts anti-tumor effect. In addition, it has the advantages of high safety and low drug resistance, when compared with other types of chemotherapy drugs, and it is associated with fewer toxic and adverse side effects [4]. In recent years, it has been reported that elemene exerts anti-tumor role by suppressing the multiplication and inducing apoptosis of carcinoma cells [5]. Clinical research has shown that phosphatase and tensin homolog deleted on chromosomal ten (PTEN) blocks the initiation of a series of downstream signal transduction pathways mediated by serine/threonine kinase (Akt), and its mechanism of action is through inhibition of Akt phosphorylation [6]. Some researchers reported that lycorine activated the intrinsic apoptosis cascade and further induced apoptosis of bladder cancer cells while blocking the Akt signaling pathway [7]. However, at present, the influence of elemene on growth and apoptotic changes in bladder cancer cells, and implication of the PTEN signal pathway have not been investigated. These formed the focus of the current study.

## EXPERIMENTAL

### Materials

Healthy human bladder cell line SV-HUC-1 was obtained from Wuxi Xinrun Biotechnology Co. Ltd., while human T24 cells were purchased from Chuanqiu Biotechnol. Company Ltd.

### Main reagents and equipment

Elemene was bought from Shanghai Yubo Biotechnology Co. Ltd. Fetal bovine serum was bought from Wuhan Purdue Life Technology Co. Ltd. Trypsin (0.25 %) was product of Shanghai Duma Biotechnology Co. Ltd. Guangzhou Yujia Biotechnology Co. was the source of MTT reagent. Western blot kit was purchased from Qiyi Biotechnology (Shanghai) Co. Ltd, while rabbit monoclonal antibodies i.e., anti-human Bcl-2, Bax, Caspase-3, PTEN, AKT, and p-AKT were purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. Mouse anti-human GRPDH monoclonal

antibody was purchased from Nanjing Wending Biomedical Technology Co. Ltd.

Thermostatic incubator was purchased from Hangzhou Notting Scientific Equipment Co. Ltd. Ultra-low temperature refrigerator was a product of Beijing Taize Jiaye Technology Development Co. Ltd., while high-speed centrifuge was purchased from Wuhan Yipu Biotechnology Co. Ltd. Fluorescent microscope was bought from Guangzhou Keshite Scientific Instrument Co. Ltd. Flow cytometer was product of Changzhou Bidaco Biotechnology Co. Ltd, while gel imaging system was purchased from PUMA Precision Medical Technology (Beijing) Co. Ltd.

### Cell grouping and treatments

The cells were routinely cultured. When cell density reached 80 – 90 %, the cells were sub-cultured and incubated in a constant temperature incubator at 37 °C in a 5 % CO<sub>2</sub> atmosphere. The T24 cells were seeded in the culture plate and randomly assigned to blank control and elemene groups. Cells in the elemene group were treated with the drug at a dose of 40  $\mu$ g/mL, while cells in blank control group were treated with an equivalent volume of DMSO. The SV-HUC-1 cells were grouped and treated in the same way as T24 cells. The two groups of cells were incubated in a constant-temperature incubator.

### Determination of cell viability

The effect of elemene on cell growth was determined with MTT method. At 24 and 48 h of incubation, 20  $\mu$ L of MTT solution was added to each well, and after further incubation for 4 h, the cell culture was subjected to centrifugation, after which PBS solution was used to rinse the cells, and MTT-containing culture solution was added. Finally, ELISA was used to determine the cell growth capacity of each group.

### Determination of apoptosis

The anti-apoptotic influence of elemene was determined flow cytometrically. Cells from each group were routinely digested, and after washing with PBS at 4 °C, the digest was centrifuged, after which the supernatant was discarded. Binding buffer was used to dilute the cells to a density of  $1 \times 10^6$  cells/mL. Then, 100  $\mu$ L of the suspension was sequentially incubated with 5  $\mu$ L of Annexin V and 1  $\mu$ L of PI solution at room temperature in the dark for 15 min. Thereafter, flow cytometry was used to

analyze the percentage of apoptosis in each group.

### Western blot assay

Western blot was used to determine the effect of elemene on expression levels of Bcl-2, Bax and caspase-3, and protein levels of PTEN, AKT and p-AKT. Total protein was extracted by lysing the cells with RIPA, and protein concentration of each lysate was determined using BCA method. Then, equal amounts of protein were resolved using SDS-PAGE, followed by transfer to PVDF membranes, and incubation with primary antibodies for Bcl-2, Bax, caspase-3, PTEN, AKT and p-AKT overnight at 4 °C. This was followed by incubation with HRP-linked secondary antibody room temperature for 2 h. The protein bands were subjected to ECL and grayscale analysis for calculation of relative expression levels.

### Statistical analysis

Measurement data are expressed as mean  $\pm$  standard deviation (SD). Comparison between two groups was done with *t*-test, while comparison amongst groups was carried out by one-way ANOVA. All statistical processing was done using SPSS23.0. Values of *p* < 0.05 were considered indicative of significant differences.

## RESULTS

### Comparison of growth between bladder cancer cells and normal bladder cells

At 24 and 48 h, the IC<sub>50</sub> of elemene in T24 cells was significantly reduced, relative to that in SV-HUC-1 cells (*p* < 0.05). These results are shown in Table 1.

**Table 1:** Comparison of IC<sub>50</sub> of elemene between bladder cancer cells and normal bladder cells

Group	24h (µg/mL)	48h (µg/mL)
SV-HUC-1	201.56±8.10	202.11±8.06
T24	43.11±6.00a	21.61±7.71a
<i>t</i>	49.708	51.175
<i>P</i> -value	<0.001	<0.001

### Percentage of apoptosis

There was higher percentage apoptosis in elemene-treated T24 cells, relative to blank control. However, percentage apoptosis was comparable in elemene-treated SV-HUC-1 cells and the blank control group (*p* > 0.05). There was a significantly higher percentage of

elemene-induced apoptosis in T24 cells than in SV-HUC-1 cells (Table 2).

**Table 2:** Degree of apoptosis in each group

Group	Treatment	Apoptosis (%)
T24	Control Blank)	4.21±0.98
	Elemene	12.05±3.15 <sup>a</sup>
SV-HUC-1	Control Blank)	2.58±0.54
	Elemene	2.95±0.68 <sup>b</sup>

<sup>a</sup>*P* < 0.05, compared with the blank control group in T24 cells; <sup>b</sup>*p* < 0.05, compared with elemene group in SV-HUC-1 cells

### Relative apoptosis-related protein levels in T24 cells

In T24 cells, the protein level of Bcl-2 in elemene group was significantly higher than the blank control value, while protein amounts of Bax and Caspase-3 were significantly reduced, relative to the corresponding levels in the blank control group (*p* < 0.05; Table 3).

**Table 3:** Expression levels of apoptosis-related proteins in T24 cells of each group

Group	Bcl-2	Bax	Caspase-3
Blank	0.31±0.15	0.86±0.10	0.89±0.06
Elemene	0.46±0.11	0.32±0.12	0.75±0.10
<i>T</i>	2.550	10.932	3.796
<i>P</i> -value	0.020	<0.001	0.001

### PTEN-AKT signal route-associated factors in T24 cells

In T24 cells, the protein expression of PTEN was significantly higher in elemene group than in blank control cells, while the expression of p-Akt protein was significantly lower. However, Akt protein expression level was comparable in both groups. These data are presented in Table 4.

**Table 4:** Protein expression levels of PTEN, AKT and p-AKT in T24 cells of each group

Group	PTEN	p-Akt	Akt
Blank control	0.45±0.05	0.68±0.07	0.92±0.04
Elemene	0.64±0.09	0.50±0.06	0.95±0.05
<i>t</i>	5.836	6.174	1.482
<i>P</i> -value	<0.001	<0.001	0.156

## DISCUSSION

Bladder cancer occurs mostly in middle-aged and old people, and its incidence is significantly higher in men than in women. Clinical literature shows that chemical substances, gene

mutations, and other factors may induce bladder cancer, and the recurrence risk of superficial bladder cancer is high, a situation that seriously impacts human health [8]. Chemotherapy is an important way of treating bladder cancer patients, and it has been widely used in clinical practice. However, most chemotherapy drugs produce significant toxic side effects as well as severe drug resistance which constitute great challenges to the treatment of bladder cancer [9].

Elemene is a new type of non-cytotoxic anti-tumor drug. Many studies reported that elemene blocks cancer cell multiplication and accelerates their apoptosis [10]. Glioma-related studies have demonstrated that elemene significantly blocked the growth and proliferation of tumor cells, and also accelerated the apoptosis of tumor cells, thereby leading to changes in amounts of apoptosis-associated proteins [11,12]. In addition, it was reported that elemene blocked the epidermal growth factor receptor signal pathway, and further enhanced the clinical effect of gefitinib in the treatment of glioblastoma [11,12]. Other studies have confirmed that elemene promoted apoptosis of bladder cancer cells and improved the antitumor effect of cisplatin in tumor, through a caspase-dependent mechanism [13]. Studies related to bladder cancer have shown that elemene exerts anti-tumor effect, and the adverse reactions caused by elemene are significantly lower, when compared with the adverse effects due to adriamycin and other drugs. Moreover, elemene does not significantly affect the quality of life of patients. However, there are limited reports on the mechanism involved in the anti-tumor effect of elemene in bladder cancer. Therefore, the present study was designed to fill this gap in knowledge.

In this study, results from MTT flow cytometric analysis showed that at 24h and 48h, the IC<sub>50</sub> of elemene in T24 cells was significantly lower than that in other cell line. In T24 cells, the percentage apoptosis was significantly higher in elemene group than in blank control. These data results suggest that elemene significantly blocked the growth of bladder cancer cells and enhanced their apoptosis. Western blot assay was used to determine the expression levels of apoptosis-related proteins in T24 cells. The Bcl-2 protein was expressed significantly higher in elemene group than in blank control group, while protein levels of Bax and Caspase-3 were significantly lower. Thus, elemene regulated the translation of apoptosis-

associated genes, leading to enhancement of the apoptosis of bladder cancer cells. The Akt signal pathway is antagonized by PTEN, and PTEN is mostly mutated or deleted in cancer cells [14]. It has been reported that the PTEN gene therapy significantly blocked Akt phosphorylation, thereby improving the cytotoxic effect of azithromycin on bladder carcinoma [15]. Other investigations demonstrated that matrine blocked cell multiplication and invasion through suppression of the expressions of cell cycle inhibitors and invasion-related genes by blocking phosphatidylinositol 3-kinase (PI3K)/AKT signal pathway. In addition, it acted synergistically with cisplatin to block urothelial carcinoma cells by down-regulating the VEGF/PI3K/AKT signal pathway.

Clinical reports indicate that PTEN is decreased in human bladder cancer tissue, but the expression level of p-Akt is significantly increased, indicating that the abnormal expressions of proteins related to the PTEN/Akt signal may be closely related to the pathogenesis of bladder cancer. The results of this study showed that in T24 cells, PTEN protein level was significantly higher in elemene group than in blank control cells, while p-Akt protein level was significantly lower than the corresponding blank control value. These data suggest that elemene inhibited bladder cancer cell growth and enhanced their apoptosis by regulating the PTEN/Akt signal pathway.

## CONCLUSION

Elemene suppresses the proliferation of bladder cancer cells and promotes their apoptosis through a mechanism involving up-regulation of the expression of PTEN and suppression of expression of p-Akt. Thus, elemene has potentials for development for the clinical treatment of bladder cancer.

## DECLARATIONS

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### **Ethical approval**

None provided.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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## REFERENCES

- Lee SH, Hu W, Matulay JT, Silva MV, Owczarek TB, Kim K, Chua CW, Barlow LJ, Kandoth C, Williams AB, et al. Tumor evolution and drug response in patient-derived organoid models of bladder cancer. *Cell* 2018; 173(2): 515-528.e17.
- Kamat AM, Sylvester RJ, Böhle A, Palou J, Lamm DL, Brausi M, Soloway M, Persad R, Buckley R, Colombel M, Witjes JA. Definitions, endpoints, and clinical trial designs for non-muscle-invasive bladder cancer: recommendations from the international bladder cancer group. *J Clin Oncol* 2016; 34(16): 1935-1944.
- van der Heijden AG, Mengual L, Ingelmo-Torres M, Lozano JJ, van Rijt-van de Westerlo CCM, Baixauli M, Geavlete B, Moldoveanu C, Ene C, Dinney CP, et al. Urine cell-based DNA methylation classifier for monitoring bladder cancer. *Clin Epigenetics* 2018; 10: 71.
- Zhang R, Pan T, Xiang Y, Zhang M, Feng J, Liu S, Duan T, Chen P, Zhai B, Chen X, et al.  $\beta$ -Elemene Reverses the Resistance of p53-Deficient Colorectal Cancer Cells to 5-Fluorouracil by Inducing Pro-death Autophagy and Cyclin D3-Dependent Cycle Arrest. *Front Bioeng Biotechnol* 2020; 8: 378.
- Xiaomeng F, Lei L, Jinghong A, Juan J, Qi Y, Dandan Y. Treatment with  $\beta$ -elemene combined with paclitaxel inhibits growth, migration, and invasion and induces apoptosis of ovarian cancer cells by activation of STAT-NF- $\kappa$ B pathway. *Braz J Med Biol Res* 2020; 53(6): e8885.
- Lee KC, Chen YL, Lin PY, Chuang WL. Ursolic acid-induced apoptosis via regulation of the PI3K/Akt and MAPK signaling pathways in Huh-7 Cells. *Molecules* 2018; 23(8): 2016.
- Díaz-Serrano A, Angulo B, Dominguez C, Pazo-Cid R, Salud A, Jiménez-Fonseca P, Leon A, Galan MC, Alsina M, Rivera F, et al. Genomic profiling of HER2-positive gastric cancer: PI3K/Akt/mTOR pathway as predictor of outcomes in HER2-positive advanced gastric cancer treated with trastuzumab. *Oncologist* 2018; 23(9): 1092-1102.
- Koutros S, Lenz P, Hewitt SM, Kida M, Jones M, Schned AR, Baris D, Pfeiffer R, Schwenn M, Johnson A, et al. RE: Elevated bladder cancer in Northern New England: The role of drinking water and arsenic. *J Natl Cancer Inst* 2018; 110(11): 1273-1274.
- Wong MCS, Fung FDH, Leung C, Cheung WWL, Goggins WB, Ng CF. The global epidemiology of bladder cancer: A join-point regression analysis of its incidence and mortality trends and projection. *Sci Rep* 2018; 8(1): 1129.
- Tang JQ, Zhao Z, Liang Y, Liao G. Robotic-assisted versus open radical cystectomy in bladder cancer: A meta-analysis of four randomized controlled trials. *Int J Med Robot* 2018; 14(1).
- Sieniawska E, Michel P, Mroczek T, Granica S, Skalicka-Woźniak K. *Nigella damascena* L. Essential oil and its main constituents, damascenine and  $\beta$ -elemene modulate inflammatory response of human neutrophils ex vivo. *Food Chem Toxicol* 2019; 125: 161-169.
- Zhou X, Cao C, Li N, Yuan S. SYL3C aptamer-anchored microemulsion co-loading  $\beta$ -elemene and PTX enhances the treatment of colorectal cancer. *Drug Deliv* 2019; 26(1): 886-897.
- Cao M, Long M, Chen Q, Lu Y, Luo Q, Zhao Y, Lu A, Ge C, Zhu L, Chen Z. Development of  $\beta$ -elemene and Cisplatin Co-Loaded Liposomes for Effective Lung Cancer Therapy and Evaluation in Patient-Derived Tumor Xenografts. *Pharm Res* 2019; 36(8): 121.
- Qiu Y, Wu Y, Meng M, Luo M, Zhao H, Sun H, Gao S. GYY4137 protects against myocardial ischemia/reperfusion injury via activation of the PHLPP-1/Akt/Nrf2 signaling pathway in diabetic mice. *J Surg Res* 2018; 225: 29-39.
- Xu T, Niu C, Zhang X, Dong M.  $\beta$ -Ecdysterone protects SH-SY5Y cells against  $\beta$ -amyloid-induced apoptosis via c-Jun N-terminal kinase- and Akt-associated complementary pathways. *Lab Invest* 2018; 98(4): 489-499.