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RESEARCH ARTICLE

ACONITINE IMPEDES CELL MOTILITY IN MDA-MB-231 BREAST CANCER CELLS

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ABSTRACT

Purpose: Aconitine, a potent alkaloid from Aconitum plants, has shown promising anticancer properties. The aim of the study is to investigate the effects of aconitine on lateral migration, and matrix metalloproteinase (MMP) activity in MDA-MB-231 triple-negative breast cancer cells.
Material and Methods: A WST-1 viability assay was conducted to determine the effect of aconitine on the viability of MDA-MB-231 cells. Following treatment with non-cytotoxic doses of aconitine, lateral migration was evaluated through wound healing assays. Additionally, gelatin zymography was conducted to analyze MMP-2 and MMP-9 activity and secretion levels.
Results: Aconitine concentrations up to 200 μM did not significantly affect cell viability for up to 72 hours, whereas higher doses (400-600 μM) reduced viability in a time-dependent manner. Aconitine at 200 μM showed a trend towards decreased lateral motility, with a significant reduction at 9 hours post-treatment. Gelatin zymography revealed no alterations in MMP-2 and MMP-9 activity or secretion levels following aconitine treatment.
Conclusion: Aconitine demonstrates limited efficacy in modulating the migratory capacity of MDA-MB-231 cells and does not affect gelatinase activity. Further investigation into underlying mechanisms is necessary, potentially leading to novel therapeutic strategies for triple-negative breast cancer.

Keywords: aconitine, cell motility, matrix metalloproteinases, triple negative breast cancer.

INTRODUCTION

According to the GLOBOCAN 2022 report, breast cancer is the most commonly diagnosed cancer among women globally, with approximately 2.3 million new cases, accounting for 11.6% of all cancer diagnoses. It is also the leading cause of cancer-related death among women, resulting in an estimated 665,000 deaths worldwide, which represents 15.4% of all cancer deaths in females. Higher incidence rates are observed in transitioned countries than those in transitioning countries; however, transitioned countries have lower mortality rates, partly due to better detection practices (1). Despite advancements in therapy options and early detection, breast cancer continues to be a significant public health challenge. Particularly triple-negative breast cancers are of critical importance due to their aggressive nature and limited treatment options, making targeted research and the development of potential therapeutic strategies essential.

Aconitine, a dominant alkaloid found in the Aconitum plants, has been widely studied for its potent

pharmacological and toxicological properties. Despite its narrow therapeutic index, aconitine is utilized in traditional Chinese medicine for various conditions such as joint pain, gastroenteritis, and rheumatoid arthritis due to its antipyretic, analgesic, and anti-inflammatory activities (2). Recent research has increasingly focused on its potential applications and risks in cancer therapy. Studies have demonstrated that aconitine exhibits promising anticancer properties by inhibiting cell proliferation, inducing apoptosis, and disrupting cancer cell signaling pathways (3). Despite its potential benefits, the high toxicity of aconitine poses significant challenges for its therapeutic application. The narrow therapeutic window and severe cardiotoxic effects necessitate careful consideration and rigorous research to harness its anticancer potential safely (2).

Matrix metalloproteinases (MMPs) are a family of calcium- and zinc-dependent endopeptidases that play a crucial role in turnover of extracellular matrix (ECM) remodeling and degradation. These enzymes are capable of cleaving various ECM components, including collagens, elastin, and proteoglycans. Moreover, MMPs can activate growth factors and cytokines, further supporting tumor growth and progression (4). They also promote tumorigenesis including inflammation, epithelial-mesenchymal transition, angiogenesis, cell migration and metastasis (4–7). Notably, gelatinases, MMP-2 and MMP-9 have been implicated in the degradation of type IV collagen, a major component of the basement membrane, thereby promoting tumor cell invasion (4,5). These findings highlight the potential of MMP inhibitors as promising anti-metastatic therapeutic agents in cancer treatment.

The aim of this study was to explore whether aconitine affects the lateral migration of MDA-MB-231 breast cancer cells and its role in modulating the secretion and activity of MMP-2 and MMP-9. This study seeks to enhance our understanding of aconitine's potential as a therapeutic agent in targeting metastasis-related processes in aggressive breast cancer phenotype.

MATERIAL AND METHODS

Ethical Considerations

Ethical approval was obtained from the Health Sciences Research Ethics Committee of Izmir University of Economics, Izmir, Turkey (Date: 12.08.2024, No: B.30.2.İEÜSB.0.05.05-20-317).

Cell Culture

Triple-negative human breast cancer cell line MDA-MB-231 (obtained from ATCC) was cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 4 mmol/L L-glutamine and 5% fetal bovine serum (FBS) (Gibco) but devoid of phenol red and antibiotics. These cells were maintained at 37°C in a humidified incubator with a 5% CO2 atmosphere. The media were changed every 2-3 days, and when the cells reached 70-80% confluency, they were detached using 0.25% Trypsin-EDTA (Gibco) for subculturing or experimentation.

Pharmacology

Aconitine (purchased from Sigma-Aldrich) was initially prepared as a 13 mM primary stock solution in ethanol (EtOH) and stored at -20°C until use. An intermediate stock solution of 2 mM aconitine was prepared by diluting the primary stock solution with the culture medium. The ethanol concentration corresponding to the highest dose of aconitine used in the experiment was utilized as the solvent control. During the experiments, the culture media were refreshed every 24 hours, regardless of whether or not they contained the added agent.

Cell Viability

WST-1 colorimetric assay was performed in order to assess the effect of aconitine on cell viability. In brief, 1 × 104 MDA-MB-231 cells were seeded into a 96-well plate and incubated for 24 hours for adherence. Cells were subsequently treated with varying concentrations of aconitine (50μM - 100μM - 200μM - 400μM - 600μM) and corresponding solvent control (4.6 % EtOH) or left untreated for 24, 48 or 72 h. At each time point, 10 μL of WST-1 reagent (Roche Diagnostics, Basel, Switzerland) was added to each well, and the plates were incubated for an additional 2 hours at 37°C in a humidified atmosphere with 5% CO2. Absorbance was measured at 450 nm, with background correction at 620 nm. The percentage of cell viability was calculated using the formula: Cel viability (%) = A treated cells/A untreated control × 100 (where A is absorbance).

Lateral Motility

The motility behavior of MDA-MB-231 cells was evaluated with wound healing assay as described previously (8). Briefly, 3x105 cells were plated in a 24-well plate and the next day pre-treated with aconitine

(200 µM), solvent control (0.77 % EtOH) or left untreated for 24h. A wound across the cell monolayer was created with a sterile 200µl micropipette tip. Following the wound creation, the cells were washed with phosphate-buffered saline (PBS) to remove any debris, and then the media with 1% FBS with agent or solvent was added to the wells. The migrated cell area was monitored at specified time intervals (0, 6, 9 and 24 h) using a microscope, and images were captured over time for the wound closure analysis. Wound widths were measured using Image J program MRI wound healing tool and lateral motility was calculated using the following equation:
Motility index, MoI (%) = [1- (wound width at given time/initial wound width)] x100.

Gelatin Zymography

MDA-MB-231 cells were treated with 200 µM aconitine or solvent control (0.77 % EtOH) in complete medium for 48 hours. Following this treatment, the cells were transferred to serum-reduced (1% FBS) media containing either the agent or the solvent for an additional 24 hours. After the incubation period, the media were collected, and loaded together with a molecular weight marker (PageRuler Pre-stained Protein Ladder, Thermo Fisher Scientific, USA) into 1 mg/mL gelatin copolymerized SDS-PAGE gels, which were then electrophoresed at 4 °C. The zymogram gels were then washed with 2.5% Triton X-100, and subsequently incubated in a development buffer composed of 50 mM Tris-base, 50 mM NaCl, 1 mM $CaCl_2$, and 0.05% Brij 35 (pH 7.6) at 37 °C for 48 hours. The gels were stained using Coomassie Blue R-250 solution and destained in a solution of 40% methanol and 10% acetic acid. The band locations for MMP-2 and MMP-9 in the gel were validated using recombinant MMP-2 and MMP-9 proteins, as previously reported (8). Gel images were captured using the UVP gel documentation system (UVP Ltd., UK), and band densities were analyzed with the UVP Bioimaging system utilizing LabWorks 8.20 Image Acquisition Software (UVP Ltd., UK). The levels of gelatinolytic activity were expressed as protease activity calculated using the formula: [area (mm^2) x optical density / µg protein].

Statistical Analysis

Data analyses were conducted using Graph Pad Prism 10.2.3 software (Graph Pad Inc, US). Data were represented as means ± standard deviations (SD). We performed the experiments in duplicate (technical replicates) and calculated the mean of each technical replicates. The mean value from each independent replicate was then used in our final statistical analysis. Consequently, our statistical calculations were based on the three mean values obtained from each independent replicate, rather than pooling all technical replicates together. First, the normality of the data distribution was confirmed with the Shapiro–Wilk test. Next, statistical significance was assessed with either two-way ANOVA, or, for unequal group sizes, the mixed-effect analysis. Tukey test or Dunnett's tests were utilized for the multiple group comparisons. Statistical significance was defined as follows: *$p < 0.05$; **$p < 0.01$

RESULTS

The effect of aconitine on MDA-MB-231 cell viability

We initially investigated the impact of varying concentrations of aconitine on the viability of MDA-MB-231 cells. The data revealed that after 24 hours, 400 µM aconitine treated-MDA-MB-231 cells exhibited a notable but not statistically significant reduction in viability (12.9% reduction) compared to the solvent control group ($p > 0.05$; $n = 3$; Figure 1). As the treatment duration increased to 48 and 72 hours, further declines in viability were observed in response to aconitine concentrations. In this context, significant decreases in viability were observed at 400 µM aconitine after both 48 h (31.9 % reduction, $p < 0.05$; $n = 3$) and 72 h (50.5 % reduction, $p < 0.05$; $n = 2$), as well as at 600 µM aconitine after 48 h (46.1 % reduction, $p < 0.01$; $n = 3$) and 72 h (76 % reduction, $p < 0.05$; $n = 2$) of exposure (Figure 1). Notably, treatment with aconitine at concentrations of up to 200 µM for periods up to 72 hours did not significantly affect the viability of MDA-MB-231 cells ($p > 0.05$; $n = 4$; Figure 1). Consequently, aconitine treatments were restricted to non-cytotoxic doses of 200 µM administered for a maximum of 72 hours, for further analysis.

Aconitine decreases MDA-MB-231 cell motility in time dependent manner

Next, we conducted a wound-healing lateral migration assay to evaluate the effect of 200 µM aconitine on the motility of MDA-MB-231 cells. As reported previously (8), we again confirmed the high motility capacity of MDA-MB-231 cells, as evidenced by an increase in the motility index (MoI) of the untreated

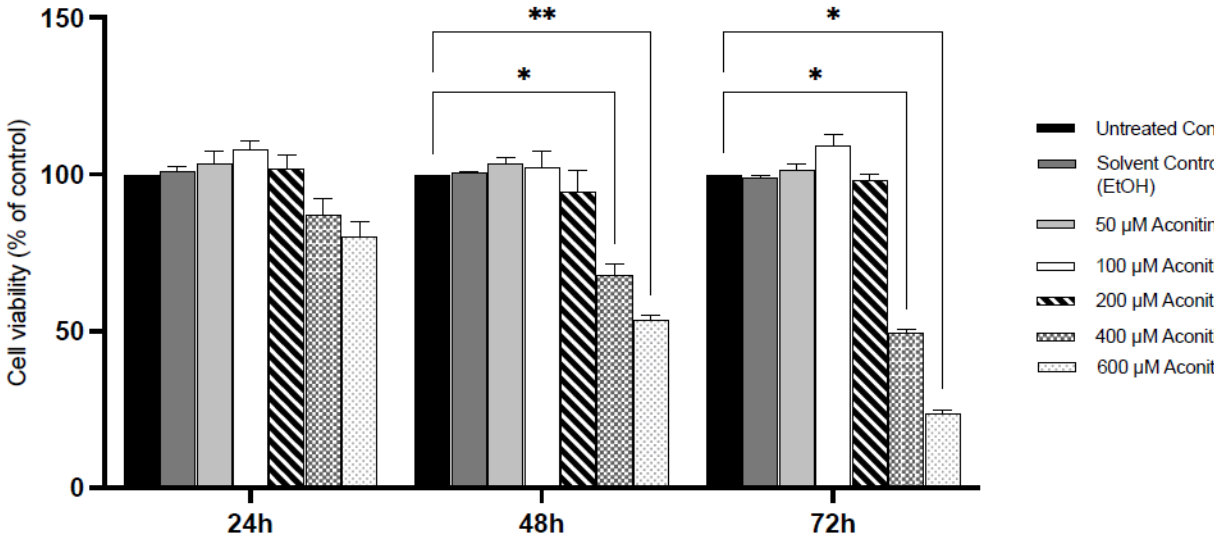

Figure 1. Effect of aconitine on MDA-MB-231 cell viability. MDA-MB-231 cells were treated with varying concentrations of aconitine (50µM - 600µM) or solvent control (4.6% EtOH) for 24, 48, and 72 hours. Cell viability was assessed using the WST-1 assay. Each bar presented as mean ± SD. *p < 0.05, **p < 0.01 compared to solvent control

control group over time, as follows: 33% at 6 h, 52% at 9 h, and 99% at 24 h (n = 3; Figure 2a and b). The solvent control (0.77% v/v EtOH) showed no significant effect on cell motility (p > 0.05; n = 3; Figure 2A and B).

Importantly, treatment with aconitine (200 µM) showed a trend towards decreased lateral motility, with MoI values of 25% (n = 3) at 6 h, 36% (n = 2) at 9 h, and 95% (n = 3) at 24 h, compared to the control groups (Figure 2A and B). Although this trend was not statistically significant overall (p > 0.05), the MoI value at 9 h was significantly decreased in aconitine-treated cells compared to the untreated control group (n = 2; p < 0.05; Figure 2A and B). These results suggest that aconitine slightly inhibits the lateral motility of MDA-MB-231 cells in a time-dependent manner.

Aconitine did not alter the activity and secretion levels of MMP-2 and MMP-9 in MDA-MB-231 cells

We also performed the gelatin zymography to investigate whether aconitine affects gelatinase activity and secretion in MDA-MB-231 cells. Gel analysis revealed that treatment with non-cytotoxic doses of aconitine (200 µM) did not alter the activity and secretion levels of either MMP-2 or MMP-9 (Figure 3A and B). This suggests that the inhibitory effect of aconitine on cellular motility may occur through an MMP-independent mechanism. However, this conclusion is based on a single biological repeat, and therefore, further replications are needed to confirm our result.

DISCUSSION

Triple-negative breast cancer (TNBC) presents significant therapeutic challenges due to the absence of hormone receptors, highlighting an urgent need for novel therapeutic strategies. The use of MDA-MB-231 cells, a well-established model for aggressive TNBC, can contribute insights into potential treatments. This study focused on the anti-migratory effects of aconitine, a potent alkaloid derived from the Aconitum plant, on the highly metastatic and aggressive TNBC MDA-MB-231 cell line. Our key findings are: i) Up to 200 µM of aconitine had no effect on MDA-MB-231 cell viability, ii) Aconitine exhibited a slight inhibitory effect on cellular motility, though overall results were not statistically significant, and iii) Non-cytotoxic doses of aconitine did not alter gelatinase secretion and activity in MDA-MB-231 cells. These suggest that aconitine may have only limited efficacy in modulating pathways associated with TNBC metastasis.

Aconitine has been characterized as a proarrhythmic agent that can open tetrodotoxin-sensitive Na+ channels, leading to persistent activation, a continuous influx of Na+, prolong the action potential and inducing cardiac arrhythmias (9-11). However, recent studies indicate that aconitine may exert effects beyond Na+ channel activation. Studies have demonstrated the aconitine and its derivatives can inhibit tumor growth by affecting various signaling pathways and inducing apoptosis in multiple cancer

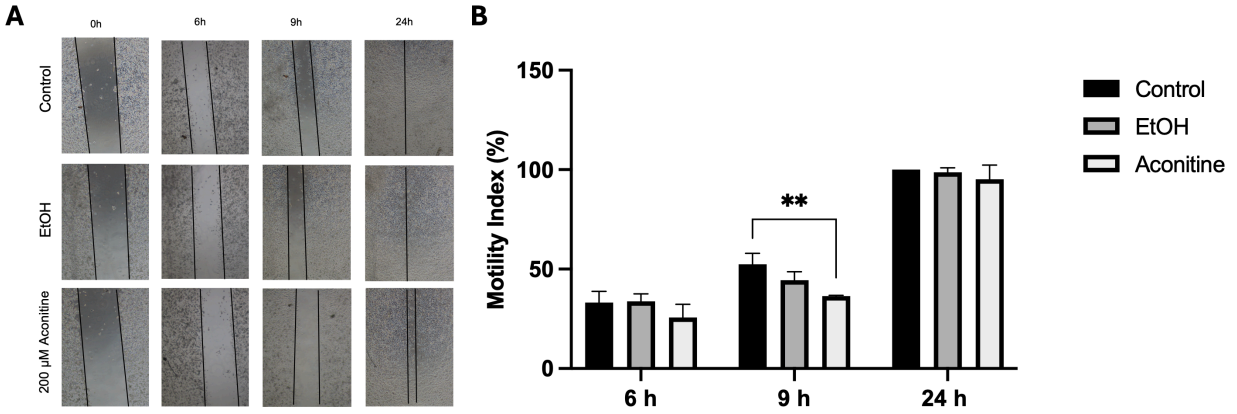

Figure 2. Impact of aconitine on lateral motility of MDA-MB-231 cells. A) Representative images of wound healing assay at 0, 6, 9, and 24 hours post-wounding (4x magnification). Cells were treated with 200 µM aconitine, solvent control (0.77% v/v EtOH), or left untreated. B) Lateral motility expressed as percentage of Motility Index (MoI%). Data are presented as mean ± SD. **p < 0.01 compared to untreated control

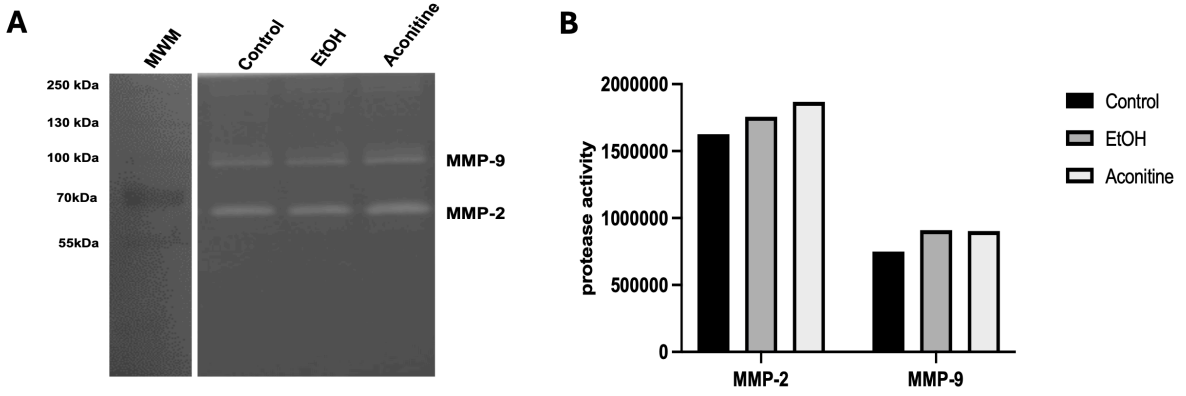

Figure 3. Effect of aconitine on MMP-2 and MMP-9 activity in MDA-MB-231 cells. A) Representative zymogram image showing gelatinase activity in conditioned media from MDA-MB-231 cells treated with 200 µM aconitine or solvent control (0.77% EtOH) for 72 hours. B) Quantification of band intensities is shown. Data are from a single biological repeat. MWM: Molecular Weight Marker

types, including pancreatic cancer (12), melanoma (13), and hepatocellular carcinoma (14).

Despite these findings, relatively few studies have reported on the effects of aconitine on cancer cell migration and invasion (15-19). For instance, aconitine has been reported to inhibit migration in MHCC97 hepatoma carcinoma cells (15). Conversely, another study revealed that aconitine increased the motility of MAT-LyLU prostate cancer cells by 15%, but had no effect on AT-2 prostate cancer cells (16). Meanwhile, Feng et al. showed that the aconitine derivative, hypaconitine, reversed induced TGF-β1 induced epithelial-mesenchymal transition (EMT) and suppressed migration and invasion in A549 lung cancer cells (17). Guo et al. found that co-treatment of aconitine with osthole and psoralen inhibited cancer cell invasion, primarily by modifying the TGF-β/Smad signaling pathway and decreasing the levels of NF-κB and RANK expression in MDA-MB-231BO breast cancer cells metastatic to bone (18). Notably, in A2780 ovarian cancer cells, aconitine decreased protein expression levels of MMP-2 and MMP-9 and cellular migration in dose dependent manner (19). Our investigation into the effect of aconitine on migration and gelatinases in TNBC cells uncovered a slight inhibition of lateral migration in MDA-MB-231 cells without a corresponding effect on the extracellular activities of MMP-2 and MMP-9.

Limitations

While our study provides valuable insights into the effects of aconitine on MDA-MB-231 cells, it is limited by insufficient replication of viability, motility and gelatin zymography assays, as well as the lack of invasion assays. These limitations necessitate further investigation to confirm the reliability of these results and clarify the potential therapeutic application of aconitine for TNBC. Additionally, since the effect of aconitine on cell viability was observed only at high doses (600 µM), we used an ethanol concentration of 4.6%, corresponding to this dose, even though it is relatively high. However, for the main experiments (zymography and motility), we applied ethanol concentrations (0.77 %) within the recommended range of 0.1-1%.

CONCLUSION

Overall, our findings suggest that aconitine may have limited efficacy in modulating the migratory capacity of the MDA-MB-231 TNBC cells, and does not affect the gelatinase activity. Future investigations should focus on four areas: i) exploring the molecular mechanisms underlying the effects of aconitine on migratory pathways, including the role of protease regulators, ii) conducting invasion assays to fully understand aconitine's impacts on metastatic behavior, iii) examining potential synergistic effects of aconitine with other anti-cancer compounds, and iv) conducting in vivo studies to elucidate the anti-metastatic potential of aconitine in a more complex physiological context.

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REFERENCES

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74(3):229–263.
2. Gao Y, Fan H, Nie A, et al. Aconitine: A review of its pharmacokinetics, pharmacology, toxicology and detoxification. J Ethnopharmacol 2022;293:115270.
3. Xiang G, Xing N, Wang S, Zhang Y. Antitumor effects and potential mechanisms of aconitine based on preclinical studies: an updated systematic review and meta-analysis. Front Pharmacol 2023;14:1172939.
4. Mustafa S, Koran S, AlOmair L. Insights Into the Role of Matrix Metalloproteinases in Cancer and its Various Therapeutic Aspects: A Review. Front Mol Biosci 2022;9:896099.
5. Conlon GA, Murray GI. Recent advances in understanding the roles of matrix metalloproteinases in tumour invasion and metastasis. J Pathol 2019;247(5):629–640.
6. Işlekel H, Oktay G, Terzi C, Canda AE, Füzün M, Küpelioğlu A. Matrix metalloproteinase-9,-3 and tissue inhibitor of matrix metalloproteinase-1 in colorectal cancer: Relationship to clinicopathological variables. Cell Biochem Funct 2007;25(4):433-441.
7. Keles D, Arslan B, Terzi C, et al. Expression and activity levels of matrix metalloproteinase-7 and in situ localization of caseinolytic activity in colorectal cancer. Clin Biochem 2014;47(13–14):1265–1271.
8. Keleş D, Sipahi M, İnanç-Sürer Ş, Djamgoz MB, Oktay G. Tetracaine downregulates matrix metalloproteinase activity and inhibits invasiveness of strongly metastatic MDA-MB-231 human breast cancer cells. Chem Biol Interact 2023;385:110730.
9. Wang SY, Wang GK. Voltage-gated sodium channels as primary targets of diverse lipid-soluble neurotoxins. Cell Signal 2003;15(2):151–159.
10. Wright SN. Comparison of aconitine-modified human heart (hH1) and rat skeletal (µ1) muscle Na+ channels: an important role for external Na+ ions. J Physiol 2002;538(Pt 3):759-771.

11. Kunze DL, Lacerda AE, Wilson DL, Brown AM. Cardiac Na currents and the inactivating, reopening, and waiting properties of single cardiac Na channels. J Gen Physiol 1985;86(5):691–719.
12. Ji BL, Xia LP, Zhou FX, Mao GZ, Xu LX. Aconitine induces cell apoptosis in human pancreatic cancer via NF-κB signaling pathway. Eur Rev Med Pharmacol Sci 2016;20:4955-4964.
13. Du J, Lu X, Long Z, et al. In Vitro and in Vivo Anticancer Activity of Aconitine on Melanoma Cell Line B16. Mol 2013:18(1):757–767.
14. Qi X, Wang L, Wang H, Yang L, Li X, Wang L. Aconitine inhibits the proliferation of hepatocellular carcinoma by inducing apoptosis. Int J Clin Exp Pathol. 2018;11(11): 5278–5289.
15. Xiong HS, Jiang C, Gao R, Chen LH. Regulatory effects and mechanism of aconitine on proliferation,invasion and migration of hepatoma carcinoma cell MHCC97. Chinese J Immunol 2018;34(5):688–692.
16. Fraser SP, Salvador V, Manning EA, et al. Contribution of functional voltage-gated Na+ channel expression to cell behaviors involved in the metastatic cascade in rat prostate cancer: I. Lateral motility. J Cell Physiol 2003;195(3):479–487.
17. Feng HT, Zhao WW, Lu JJ, Wang YT, Chen XP. Hypaconitine inhibits TGF-β1-induced epithelial–mesenchymal transition and suppresses adhesion, migration, and invasion of lung cancer A549 cells. Chin J Nat Med 2017;15(6):427-435.
18. Guo BF, Liu S, Ye YY, Han XH. Inhibitory effects of osthole, psoralen and aconitine on invasive activities of breast cancer MDA-MB-231BO cell line and the mechanisms. Zhong Xi Yi Jie He Xue Bao 2011;9(10):1110–1117.
19. Wang X, Lin Y, Zheng Y. Antitumor effects of aconitine in A2780 cells via estrogen receptor β-mediated apoptosis, DNA damage and migration. Mol Med Rep 2020;22(3):2318-2328.