Journal of Cardiothoracic Surgery

RESEARCH

Open Access

Aconitine promotes ROS-activated P38/MAPK/Nrf2 pathway to inhibit autophagy and promote myocardial injury

Chunai Yang[1], Jinxiao Fu[2*], Fenshuang Zheng[1], Yangshan Fu[1], Xueqiong Duan[1], Ruiling Zuo[1] and Junbo Zhu[1]

Abstract

Background Aconitine has cardiotoxicity, but the mechanism of cardiotoxicity induced by aconitine is limited. The aim of this study was to investigate the mechanism of myocardial injury induced by aconitine.

Methods Using aconitine, ROS inhibitor N-acetylcysteine(NAC), the autophagy activitor Rapamycin (Rap) or the P38/MAPK pathway activitor Dehydrocorydaline treats H9C2 cells. CCK-8 assay was used to assay cell proliferation activity. Flow Cytometry was used to detect cell apoptosis. Dichloro-dihydrofluorescein diacetate was used to detect ROS levels. The expression of LC3 was detected by Immunofluorescence Staining. Western blotting detected the expression of related proteins. The mRNA levels of inflammatory factors were detected by RT-qPCR.

Results Aconitine inhibits cardiomyocyte proliferation, induces apoptosis and secretion of inflammatory factors. Aconitine activates the P38/MAPK/Nrf2 pathway, induces ROS increase, and promotes autophagy. NAC can inhibit proliferation inhibition, apoptosis, inflammation and P38/MAPK/Nrf2 pathway activation induced by aconitine. Rap and P38 activators can partially recover the effects of NAC on proliferation, apoptosis, inflammation and autophagy of cardiomyocytes.

Conclusion Aconitine promotes ROS-activated P38/MAPK/Nrf2 pathway to inhibit autophagy and promote myocardial injury.

Keywords Aconitine, ROS, Autophagy, P38/MAPK/Nrf2 pathway, Myocardial injury

Introduction

Aconitine, a diterpenoid alkaloid predominantly found in Aconitum species such as Aconitum and Aconitum kongboense, is widely recognized in traditional Chinese medicine for its potent pharmacological properties [1]. It has demonstrated potential therapeutic benefits in treating a variety of diseases, including cancer, systemic lupus erythematosus, and rheumatoid arthritis [2–4]. However, aconitine is also a highly toxic compound, with pronounced cardiotoxicity and neurotoxicity [5]. The cardiotoxicity of aconitine is primarily characterized by arrhythmias, hypotension, palpitations, and heart failure, which, in severe cases, can be fatal. This toxicity presents a major limitation to the clinical application of aconitine [6, 7]. Therefore, it is essential to thoroughly investigate the mechanisms underlying aconitine-induced cardiotoxicity to improve its safety and therapeutic potential.Oxidative stress plays a major role in various drug-induced toxicities [8]. Cardiotoxicity has been associated with an

*Correspondence:
Jinxiao Fu
yangchunai2020@163.com
[1] Department of Emergency, The Affiliated Hospital of Yunnan University, Kunming 650021, China
[2] Department of Geriatric Medicine, The Affiliated Hospital of Yunnan University, 176 Qingnian Road, Wuhua District, Kunming 650021, Yunnan, China

imbalance of reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels, leading to subcellular structural damage and cell death due to insufficient antioxidant defenses [9]. Numerous studies have shown that aconitine-induced damage to H9c2 cells is caused by increased oxidative stress, which triggers apoptosis and autophagy, disrupts mitochondrial membrane potential, and induces Ca2+ overload [10]. Intracellular calcium overload can enhance ROS production, leading to further apoptosis or autophagy [10]. Similarly, doxorubicin is known to cause cardiotoxicity by increasing ROS levels [11]. However, the specific mechanism by which aconitine enhances ROS expression and promotes cardiotoxicity remains elusive.

The MAPK signaling pathway, a critical component of the eukaryotic signal transduction network, plays a pivotal role in cell proliferation, differentiation, apoptosis, and stress response under both normal and pathological conditions [12]. This pathway can be divided into three major sub-pathways: ERK1/2, p38, and JNK [13]. Among these, p38 MAPK has emerged as a key target for preventing and treating inflammation and autophagy, as it is frequently activated by environmental stress and cytokines, contributing to inflammation, oxidative stress, and autophagy [14, 15]. Oxidative stress can induce receptor-dependent apoptosis and damage mitochondria in healthy cells [16]. This mitochondrial dysfunction leads to ROS accumulation, which subsequently stimulates the p38 MAPK pathway [17]. Therefore, we hypothesize that aconitine-induced cardiotoxicity may be due to the activation of the ROS/p38 MAPK pathway. In this study, we found that aconitine-induced myocardial injury and autophagy are closely associated with oxidative stress, specifically through the activation of the ROS-regulated P38 MAPK signaling pathway. We then tested whether inhibiting these pathways could potentially alleviate aconitine-induced myocardial injury and autophagy. To explore this hypothesis, we treated the cells with the ROS inhibitor NAC, the autophagy activator Rapamycin (Rap), and a P38 MAPK activator. Our findings revealed that aconitine promotes ROS-mediated activation of the P38/MAPK/Nrf2 pathway, which inhibits autophagy and exacerbates myocardial injury. This discovery suggests a novel approach for developing combination therapies that incorporate cardioprotective strategies in clinical applications involving aconitine.

Method and material

Cell culture

H9C2 immortalized rat cardiomyocytes were acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in DMEM (Gibco, USA) supplemented with 10% FBS (HyClone, USA), 100 units/mL penicillin, and 100 μg/mL streptomycin (Solarbio, China), and maintained at 37 °C in a 5% CO2. The cells used in this article did not show Mycoplasma infection. And it has been certified by STR.

Treatment of cells

The experiment was conducted in accordance with the following groups: NC group, aconitine group, NAC group, NAC + Rap group, and NAC + P38 MAPK pathway activator group(NAC + P38 activator group). Aconitine(Selleck Chemicali, China) was allocated to a concentration range of 0, 0.25, 0.5, 1, and 2 μM. Cells were treated with the aconitine solution for 24 h and cell viability was assessed. The optimal concentration for the model was identified. NC group was H9C2 cells in normal culture, aconitine group was H9C2 cells treated with 1 μM aconitine for 24 h, NAC group was H9C2 cells treated with 1 μM aconitine and then with 10 mM ROS inhibitor N-acetylcysteine [18] (NAC, Sigma, USA), NAC + Rap group was H9c2 cells treated with 1 μM aconitine and then with 10 μM rapamycin (Rap, Sigma, USA), and NAC + P38 activator group was H9C2 cells treated with 1 μM aconitine and then with 10 μg/mL P38 MAPK pathway activator Dehydrocorydaline (DHC, MCE, USA).

Cell viability assay

The cells were seeded into 96-well plates and treated with aconitine, NAC, rapamycin, or a P38 activator according to the experimental groups. Cell viability was then assessed using the CCK-8 assay kit (Jiancheng, China) following the manufacturer's instructions.. Specifically, 10 μL of the CCK-8 reagent was added to the culture medium in each well, and the plate was incubated in the dark at 37 °C for 3 h. Absorbance was measured at 450 nm using a microplate reader (BioTek, USA).

Flow cytometry analysis

The apoptotic rate of the cells was assessed using an Annexin V-FITC/PI apoptosis detection kit (Beyotime, China). First, the cells were treated according to the

Table 1 Primers used for RT-qPCR

Gene		Primer size	Primer sequences (5′-3′)
IL-1β	FORWARD	95	TCTCACAGCAGCATCTCGACAAG
IL-1β	REVERSE	95	CCACGGGCAAGACATAGGTAGC
TNF-α	FORWARD	104	CCGAGATGTGGAACTGGCAGAG
TNF-α	REVERSE	104	CCACGAGCAGGAATGAGAAGAGG
IL-6	FORWARD	94	GCCTTCTTGGGACTGATGTTGTTG
IL-6	REVERSE	94	GTCTGTTGTGGGTGGTATCCTCTG

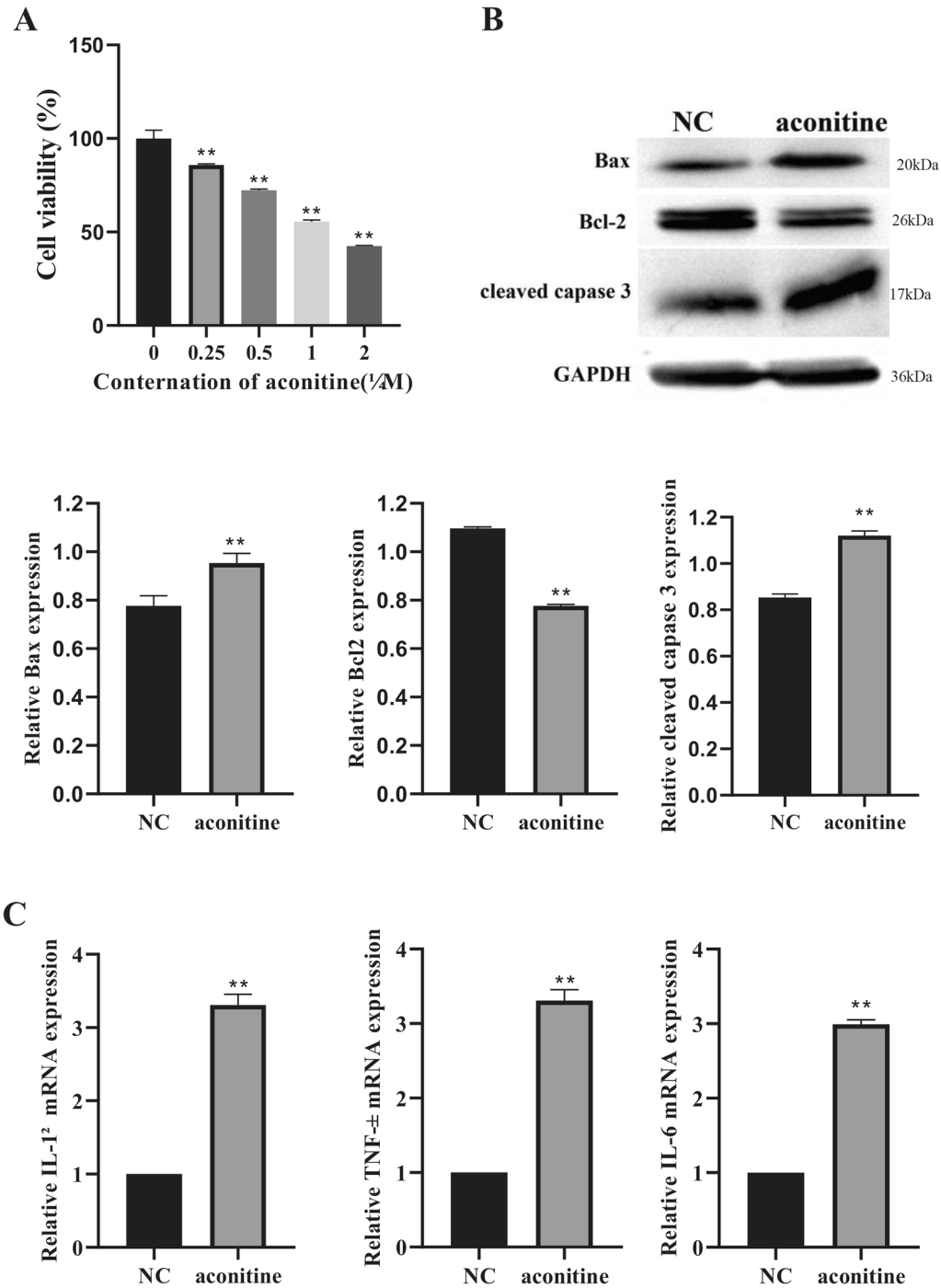

Fig. 1 Aconitine damages cardiomyocyte injury cells. **A** CCK-8 was used to detect cell viability. **B** Western blotting was used to detect apoptosis-related proteins. **C** The expression of IL-1β, TNF-α and IL-6 was detected by RT-qPCR. All values represent means ± SD (n = 3 biological replicates). $P < 0.01$ (**)

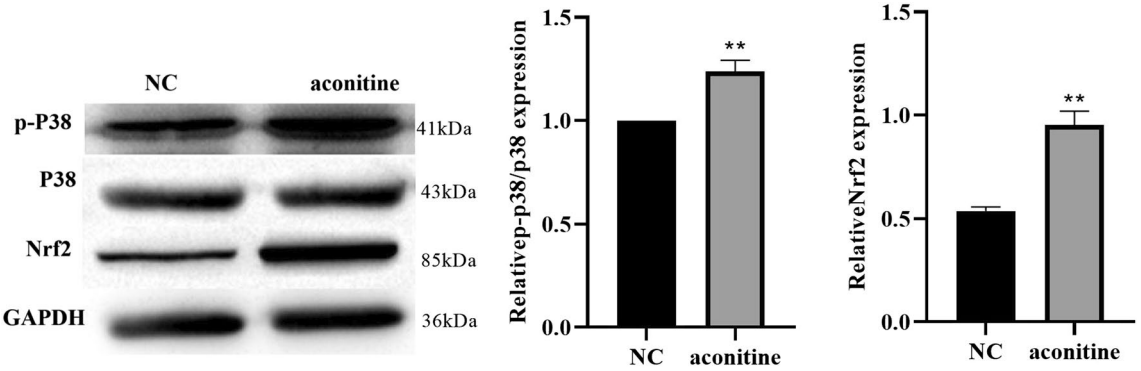

Fig. 2 Aconitine activates the P38/MAPK/ Nrf2 pathway. Western blotting was used to detect the P38/MAPK/ Nrf2 pathway-related proteins. All values represent means ± SD (n = 3 biological replicates). $P < 0.01$ (**)

experimental groups. Afterward, 5×10^5 cells were collected and resuspended in 100 μL of 1 × binding buffer. Then, 5 μL of Annexin V and 5 μL of PI working solution were added to each tube, and the cells were incubated in the dark for 15 min. Finally, the apoptotic rate was measured using a flow cytometer (BD, USA) with an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

Detection of ROS levels by immunofluorescence

Intracellular ROS levels were detected using the fluorescent probe dichloro-dihydrofluorescein diacetate (DCFH-DA) (Sigma, USA). The cells were initially treated according to their groupings and then subjected to incubation with 50 μM DCFH-DA at 37 °C in darkness for 30 min. Following this, the cells were washed twice with cold PBS (Sigma, USA). Fluorescence images of the intracellular ROS were subsequently captured using a fluorescence microscope (Olympus a clean version without tracked changes or highlights, Japan).

Immunofluorescence staining

After the cells were processed according to the grouping information, they were washed three times with PBS. Then H9C2 cells were fixed with 4% paraformaldehyde (Sigma, USA) for 30 min, washed with PBS for 3 times, and then treated with 0.2% Triton X-100 (Solarbio, China) for 20 min at room temperature. After washing with PBS for 3 times, the cells were blocked in 5% BSA (Servicebio, China) for 30 min at room temperature. Subsequently, the cells were incubated with LC3 primary antibody (CST, USA, Rabbit mAb, 1:1600) at 4 °C overnight. After that, the sample was incubated with FITC-conjugated secondary antibody (abcam, UK, Goat Anti-Rabbit IgG H&L (FITC), 1:5000) at 37° C overnight. The cells were then stained with DAPI (Wuhan Antgene, China) for 5 min. The images were taken using a fluorescence microscope.

Western blotting

Cell protein extraction was performed using a protein extraction kit from Thermo Fisher Scientific (USA). The cells were homogenized with RIPA protein extraction reagent, and the homogenate was separated by SDS-PAGE (Thermo Fisher Scientific, USA) before being transferred to a PVDF membrane (Millipore, USA). After blocking the membrane with 5% skim milk (Beyotime, China), primary antibodies were incubated at 4 °C overnight. The membrane was then incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature for 2 h. Protein bands were visualized using ECL (Thermo Fisher Scientific, USA) on a chemiluminescent imaging system (Bio-Rad, USA), and the grayscale values of the target bands were quantified using ImageJ. The primary antibodies used were Bax (ABclonal, China, Rabbit mAb, 1:2000), Bcl2 (CST, USA, Rabbit mAb, 1:1000), cleaved caspase-3 (CST, USA, Rabbit pAb, 1:1000), P62 (abcepta, China, Rabbit pAb, 1:2000), LC3 (ABclonal, China, Rabbit pAb, 1:1000)), Beclin 1 (CST, USA, Rabbit mAb, 1:1000), p-p38 (CST, USA, Rabbit mAb, 1:1000), p38 (CST, USA, Rabbit pAb, 1:1000), and Nrf2 (CST, USA, Rabbit mAb, 1:1000). The secondary antibody used was goat anti-rabbit IgG (heavy and light chain) (CST, USA, 1:5000).

RT-qPCR

Total RNA was isolated from cells by using TRIzol reagent (Invitrogen, USA), which was reverse transcribed into cDNA using HiScript® II Q RT SuperMixfor qPCR Kit (Vazyme; China). After this, Taq Pro Universal SYBR qPCR Master Mix (Vazyme; China) was used for qPCR

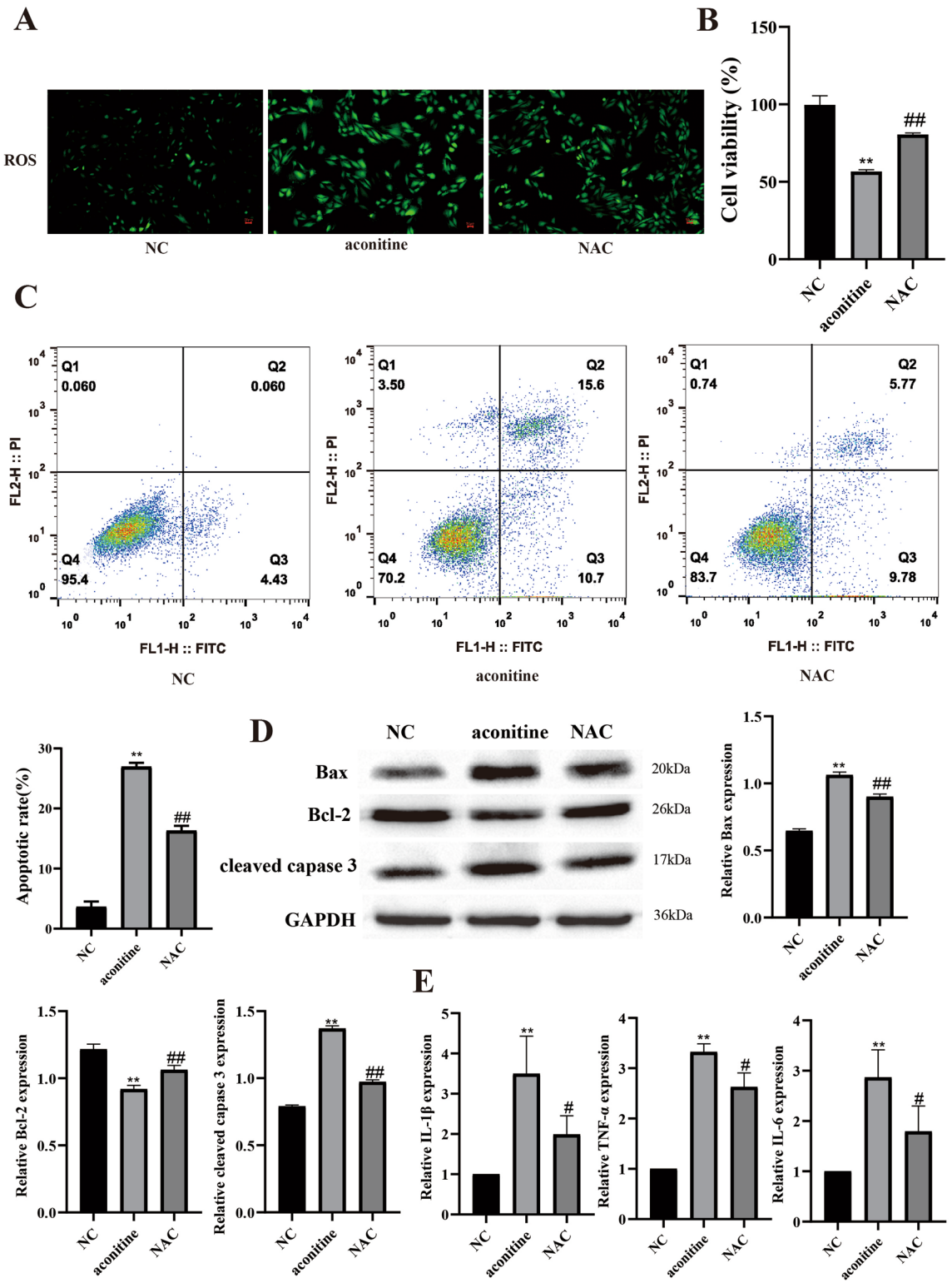

Fig. 3 Aconitine exacerbates myocardial cell damage through ROS activation. **A** ROS was detected by immunofluorescence. Scale bar = 50 μm. **B** CCK-8 was used to detect cell viability. **C** Apoptosis was detected by flow cytometry. **D** Western blotting was used to detect apoptosis-related proteins. **E** The expression of IL-1β, TNF-α and IL-6 was detected by RT-qPCR. All values represent means ± SD (n = 3 biological replicates). Compared with NC group, $P < 0.01$ (**). $P < 0.05$ (*); Compared with aconitine, $P < 0.01$ (##). $P < 0.05$ (#)

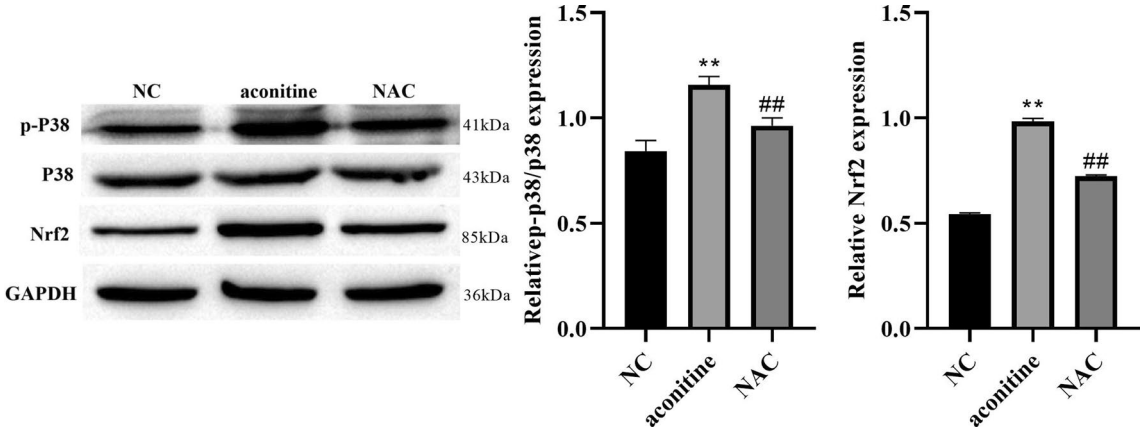

Fig. 4 Aconitine activates the P38/MAPK/ Nrf2 pathway through ROS. Western blotting was used to detect the P38/MAPK/ Nrf2 pathway-related proteins. All values represent means ± SD (n = 3 biological replicates). Compared with NC group, $P < 0.01$ (**). $P < 0.05$ (*); Compared with aconitine, $P < 0.01$ (##). $P < 0.05$ (#)

with GAPDH as an internal reference. The relative gene expression levels were then calculated using the $2^{-\Delta\Delta Ct}$ method. The primers (QingKe; China) used are listed in Table 1.

Statistical analysis

In this study, each experiment was conducted with three biological replicates. Data are expressed as mean ± standard deviation (SD). Comparisons between two groups were made using the Student's t-test (two-tailed). Comparisons between three or more groups were performed using one-way ANOVA followed by Tukey's post hoc test. A significance level of $P < 0.05$ was considered statistically significant. The software used was GraphPad Prism Version 8.0.2 (GraphPad,USA).

Results

Aconitine induces injury in cardiomyocytes

To investigate the impact of aconitine on H9C2 cells, the effects of varying concentrations of aconitine (0, 0.25, 0.5, 1, and 2 μM) on cell viability were examined using the CCK-8 assay after a 24-h exposure period (Fig. 1A). Aconitine significantly decreased cell viability in a dose-dependent manner. In response to this observation, Western blot analysis was performed to further explore the cellular mechanisms, revealing a significant upregulation of the apoptosis-related proteins Bax and cleaved caspase-3, along with a downregulation of the anti-apoptotic protein Bcl-2 (Fig. 1B). Additionally, aconitine exposure led to a significant increase in the mRNA expression levels of the pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 (Fig. 1C). These results suggest that aconitine exerts detrimental effects on H9C2 cells, likely through the induction of apoptosis and inflammation.

Aconitine activates the P38/MAPK/ Nrf2 pathway

This study aimed to investigate the effects of aconitine on the P38/MAPK/Nrf2 pathway in H9C2, utilizing Western blotting to observe a significant increase in the expression of p-P38 and Nrf2 (Fig. 2). This finding suggests that aconitine may activate the P38/MAPK/Nrf2 signalling pathway in the H9C2 cells.

Aconitine promotes cardiomyocyte injury through ROS

Research has established a strong link between ROS production and the P38/MAPK/Nrf2 signaling pathway, but the effects of aconitine on ROS generation in cell lines remain unclear. Immunofluorescence imaging demonstrated that aconitine treatment significantly elevated cellular ROS levels (Fig. 3A). In contrast, the antioxidant NAC reduced ROS production (Fig. 3A), improved cell viability, and decreased apoptosis compared to the aconitine-treated group (Fig. 3C). Furthermore, NAC successfully reversed aconitine-induced changes in the expression of apoptosis-related proteins and inflammatory factors (Fig. 3D and E). Overall, NAC shows potential as an effective agent for counteracting the harmful effects of aconitine on cardiomyocytes.

Aconitine activates the P38/MAPK/Nrf2 pathway through ROS

Previous studies suggest that aconitine may damage cardiomyocytes by increasing ROS. However, whether ROS directly activates the P38/MAPK/Nrf2 signaling pathway

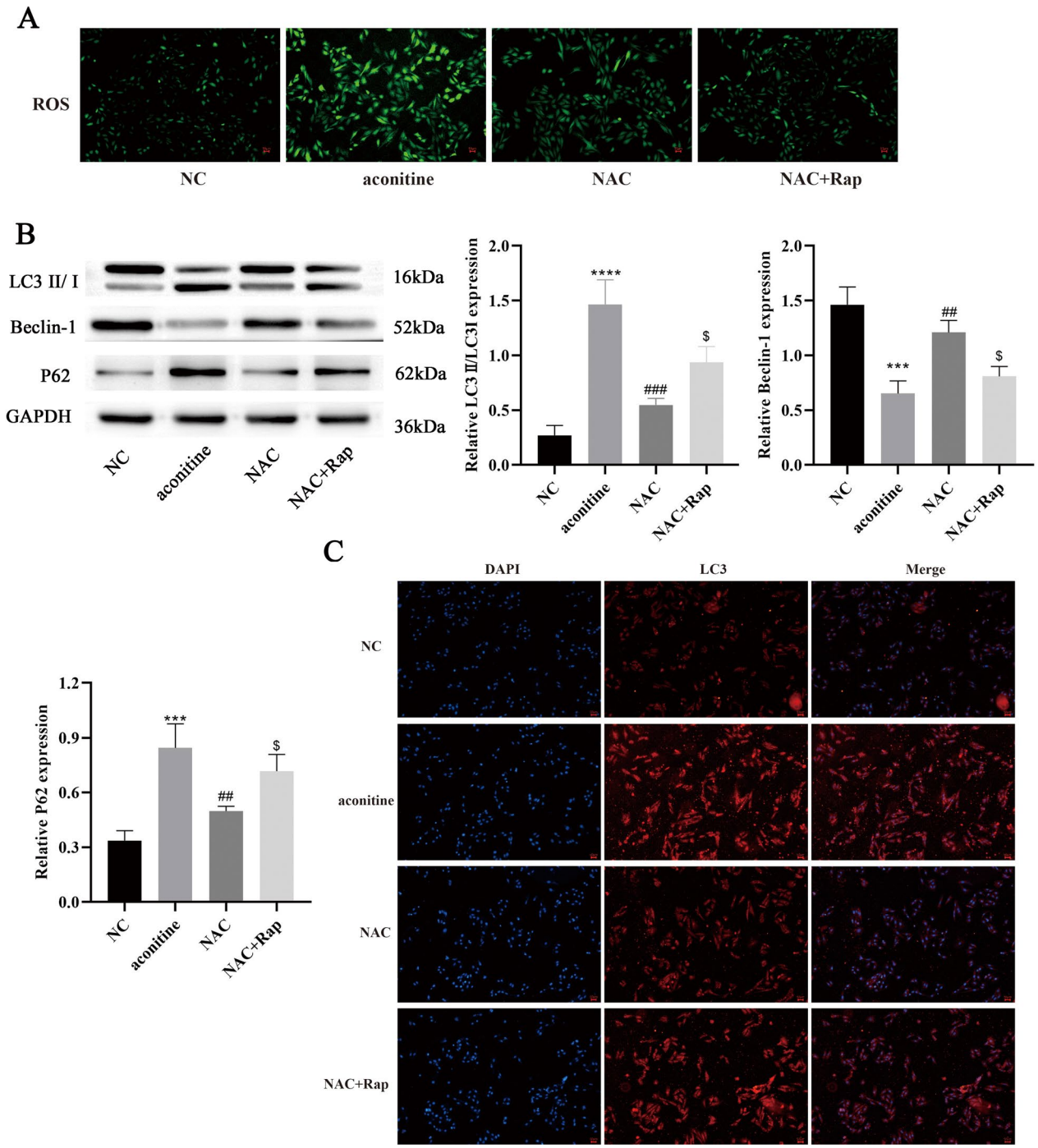

Fig. 5 Aconitine promotes cardiomyocyte injury by inhibiting autophagy through ROS. **A** ROS was detected by immunofluorescence. Scale bar = 50 μm. **B** Western blotting was used to detect LC3 II/LC3 I, Beclin-1, and P62. C: LC3 was detected by immunofluorescence. Scale bar = 50 μm. All values represent means ± SD (n = 3 biological replicates). Compared with NC group, $P < 0.01$ (**). $P < 0.05$ (*); Compared with aconitine, $P < 0.01$ (##). $P < 0.05$ (#); Compared with NAC, $P < 0.01$ ($$). $P < 0.05$ ($)

remains uncertain. In our further investigation, NAC was shown to reduce the expression levels of p-P38/P38 and Nrf2 proteins compared to the aconitine group (Fig. 4). These findings indicate that aconitine may indeed activate the P38/MAPK/Nrf2 pathway through ROS, ultimately leading to cardiomyocyte damage.

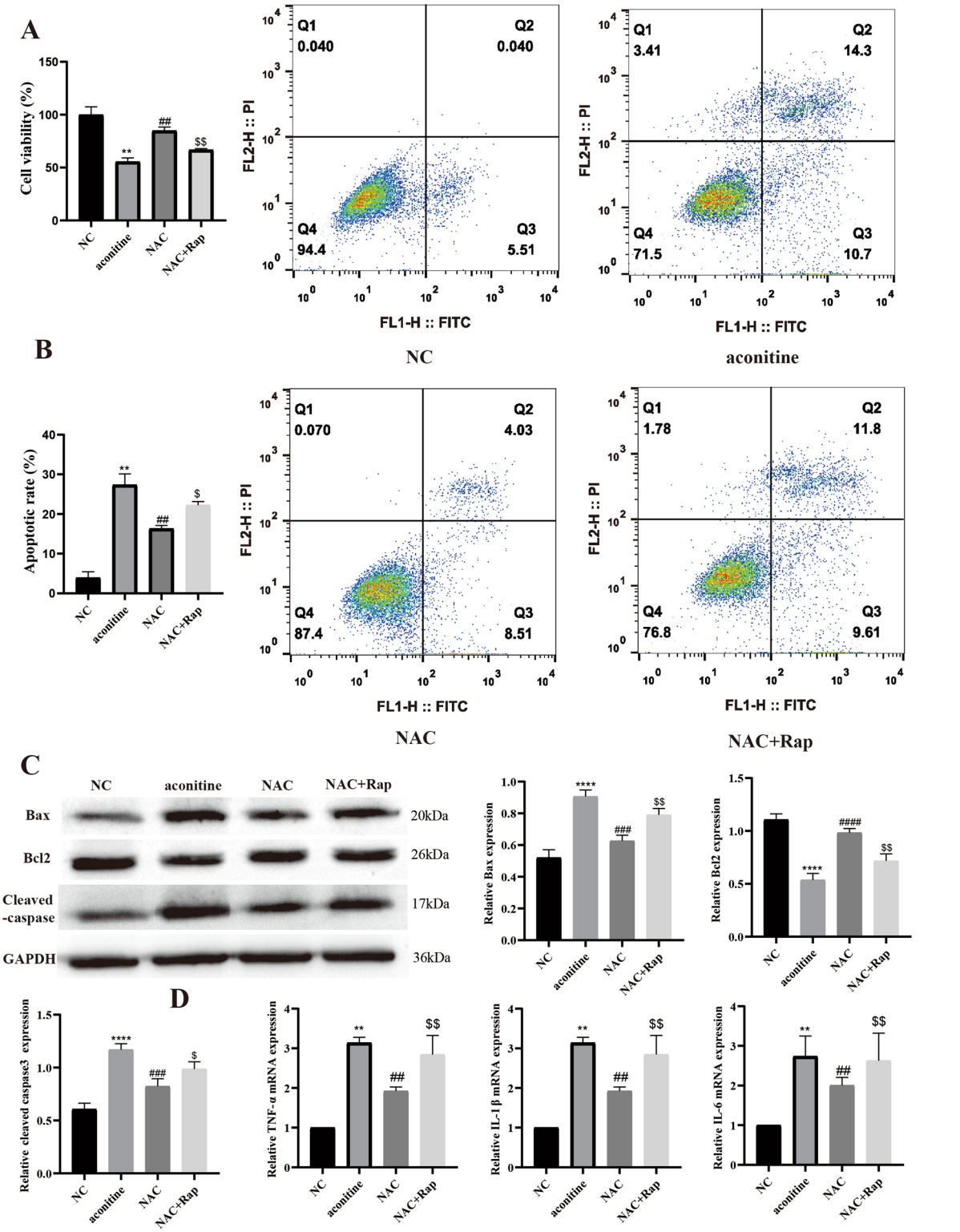

Fig. 6 Aconitine promotes cardiomyocyte injury by inhibiting autophagy through ROS. **A** CCK-8 was used to detect cell viability. **B** Apoptosis was detected by flow cytometry. **C** Western blotting was used to detect apoptosis-related proteins. **D** The expression of IL-1β, TNF-α and IL-6 was detected by RT-qPCR. All values represent means ± SD (n = 3 biological replicates). Compared with NC group, $P < 0.01$ (**). $P < 0.05$ (*); Compared with aconitine, $P < 0.01$ (##). $P < 0.05$ (#); Compared with NAC, $P < 0.01$ ($$). $P < 0.05$ ($)

Aconitine induces cardiomyocyte injury by promoting autophagy via ROS

This study aimed to investigate the relationship between aconitine-induced myocardial autophagy and ROS. We treated cells with aconitine, followed by N-acetylcysteine (NAC) or a combination of NAC and rapamycin (Rap). Our results show that NAC effectively inhibits aconitine-induced ROS production. Interestingly, adding Rap to NAC did not further reduce ROS levels compared to NAC alone (Fig. 5A). Western blot analysis demonstrated that NAC decreased the levels of autophagy-associated proteins LC3 II/LC3 I and P62 while increasing Beclin-1 expression. However, Rap partially reversed the effects of NAC (Fig. 5B). Additionally, immunofluorescence experiments confirmed that NAC reduced LC3 expression, whereas Rap increased it (Fig. 5C). These findings suggest that the autophagy-promoting effect of ROS induced by aconitine may contribute to its cardiotoxicity.

Aconitine promotes cardiomyocyte injury by inhibiting autophagy through ROS

We conducted a cell viability assay using the CCK-8 reagent and found that NAC significantly increased cell survival compared to the aconitine group, while Rap reversed this effect (Fig. 6A). To further investigate the impact on apoptosis, we employed flow cytometry and protein blotting. Flow cytometry showed that NAC markedly reduced apoptosis in cardiomyocytes compared to the aconitine group, whereas Rap negated this effect (Fig. 6B). Similarly, protein blotting confirmed that NAC inhibited aconitine-induced apoptosis (Fig. 6C). Additionally, quantitative real-time PCR (RT-qPCR) was used to measure the mRNA levels of inflammatory factors IL-1β, TNF-α, and IL-6. The results indicated that NAC effectively decreased the expression of these inflammatory factors, while Rap reversed this reduction (Fig. 6D).

Aconitine promotes myocardial apoptosis and inflammation through the ROS-activated P38/MAPK/Nrf2 pathway

This study aimed to determine whether aconitine-induced myocardial injury is linked to ROS activation of the P38/MAPK/Nrf2 signaling pathway. To explore this, we treated cells with a P38/MAPK pathway activator. Western blot analysis revealed a significant increase in P38 and Nrf2 expression in the NAC + P38 activator group compared to the NAC group (Fig. 7A). Immunofluorescence analysis indicated that the P38 activator had no significant effect on ROS levels (Fig. 7B). However, the P38 activator inhibited cell proliferation (Fig. 7C) and promoted apoptosis (Fig. 7D). Protein blotting further confirmed that the NAC + P38 activator group showed significantly lower expression of Bax and cleaved caspase-3, along with significantly higher expression of Bcl2, compared to the NAC group (Fig. 7E). Additionally, the expression of inflammatory factors increased following treatment with the P38 activator (Fig. 7F). These findings suggest that aconitine promotes myocardial apoptosis and inflammation through the ROS-activated P38/MAPK/Nrf2 pathway.

Aconitine promotes myocardial autophagy through the ROS-activated P38/MAPK/Nrf2 pathway

This study aimed to explore whether aconitine influences autophagy through the ROS-activated P38/MAPK/Nrf2 signaling pathway. After treating cells with a P38/MAPK activator, we observed a significant increase in LC3I/II and Beclin-1 expression levels, accompanied by a marked decrease in P62 expression (Fig. 8A). These findings were corroborated by the immunofluorescence analysis of LC3, which showed consistent results with the protein expression data (Fig. 8B). Overall, these results indicate that aconitine stimulates myocardial autophagy via the ROS-activated P38/MAPK/Nrf2 pathway.

Discussion

Aconitine, the primary active component of Aconitum, has gained significant attention for its potent anti-inflammatory and analgesic properties, as well as its potential as an anti-tumor and cardiotonic agent [19]. However, aconitine is also known for causing unpredictable cardiotoxicities, which can vary widely among individuals [20]. The cardiac effects of aconitine has been extensively studied in previous research [20–22]. For example, aconitine has been shown to inhibit PC12 cell proliferation in a dose-dependent manner, increase apoptosis, [23] and trigger inflammation [24]. Similarly, in our study, we observed

(See figure on next page.)

Fig. 7 Aconitine promotes cardiomyocyte injury by inhibiting autophagy through ROS. **A** Western blotting was used to detect P38/MAPK/Nrf2 pathway-related proteins. **B** ROS was detected by immunofluorescence. Scale bar = 50 μm. **C** CCK-8 was used to detect cell viability. **D** Apoptosis was detected by flow cytometry. **E** Western blotting was used to detect apoptosis-related proteins. **F** The expression of IL-1β, TNF-α and IL-6 was detected by RT-qPCR. All values represent means ± SD (n = 3 biological replicates). Compared with NC group, $P < 0.01$ (**). $P < 0.05$ (*); Compared with aconitine, $P < 0.01$ (##). $P < 0.05$ (#); Compared with NAC, $P < 0.01$ ($$). $P < 0.05$ ($)

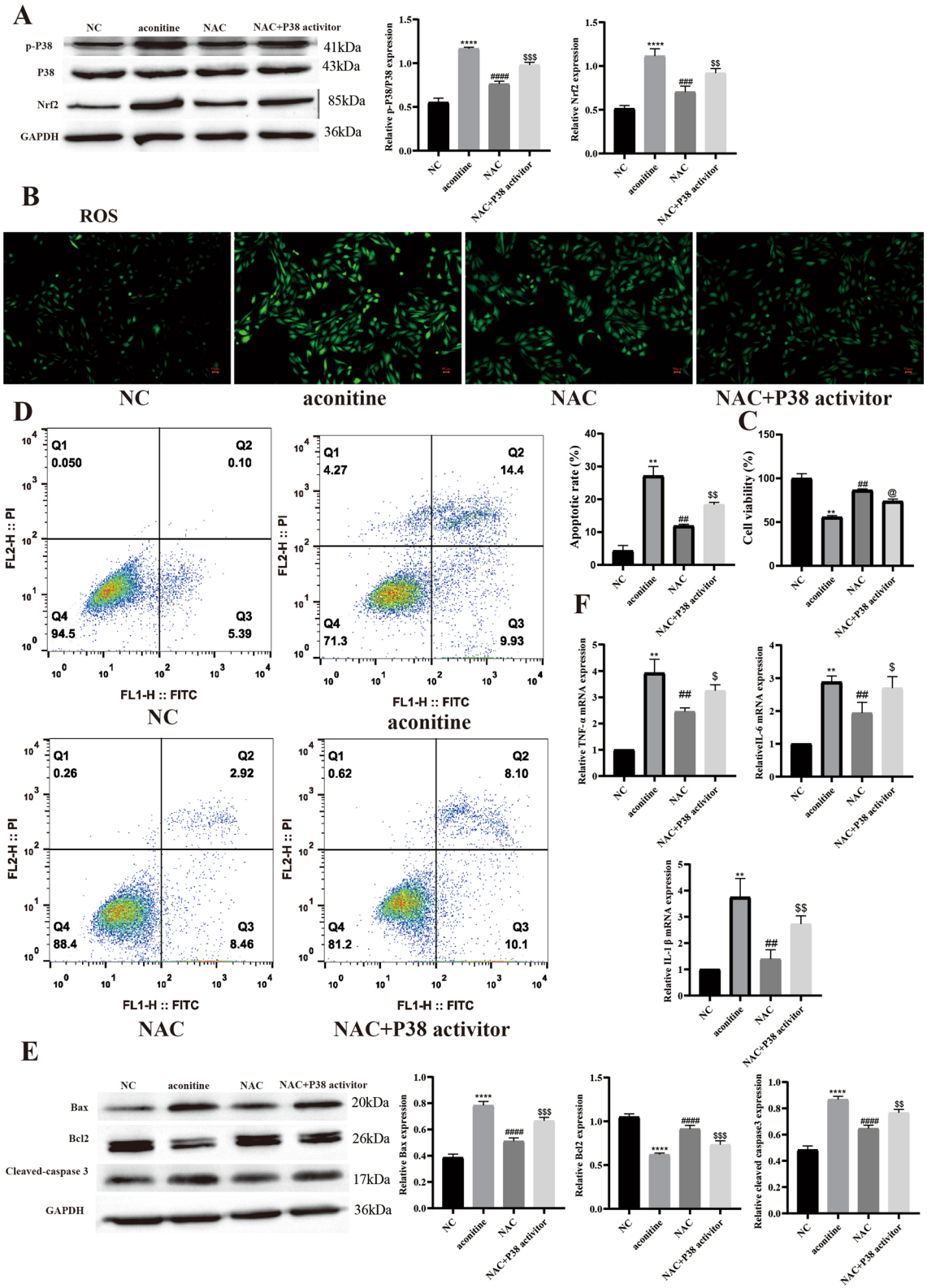

Fig. 7 (See legend on previous page.)

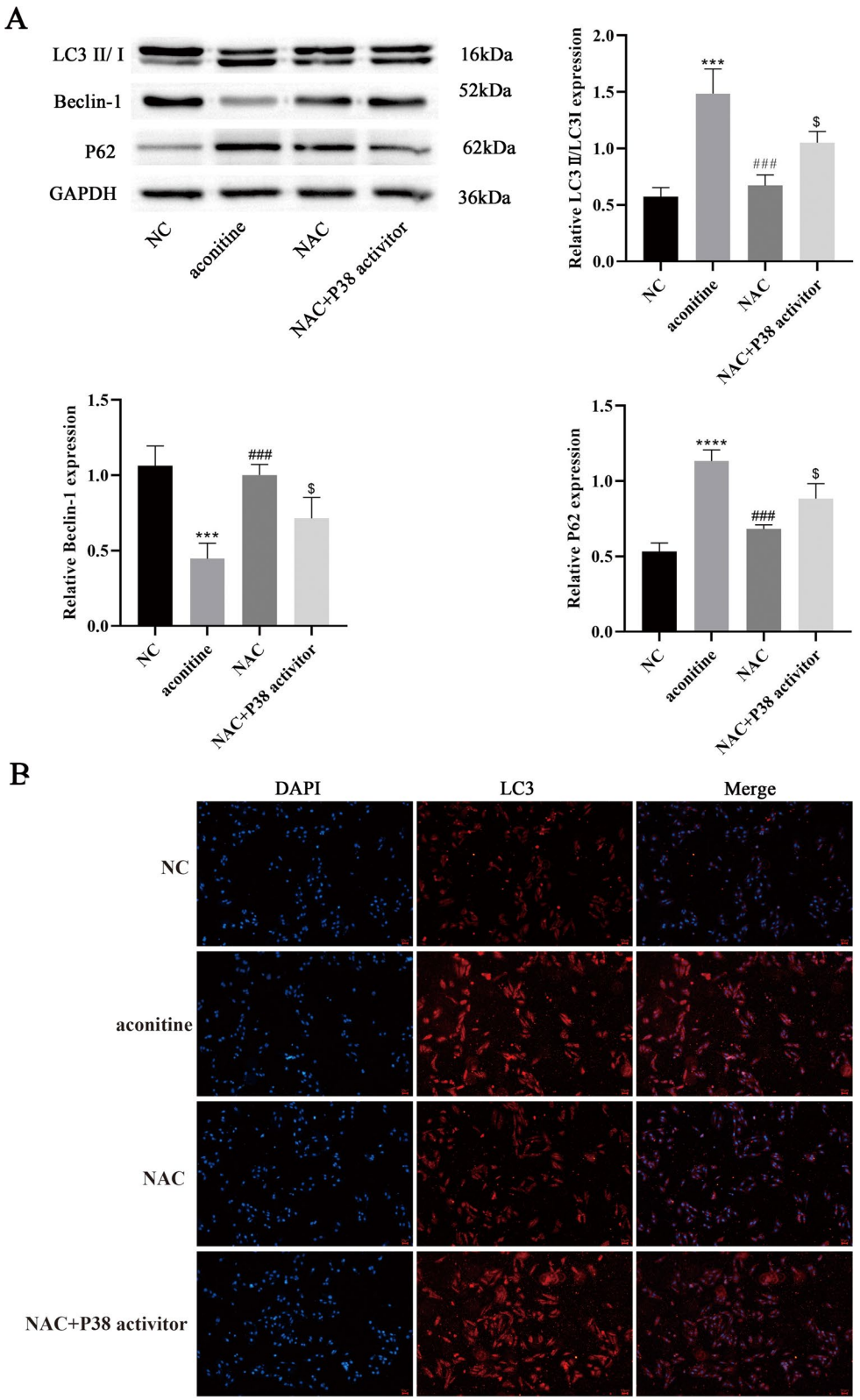

Fig. 8 Aconitine promotes myocardial autophagy through the ROS-activated P38/MAPK/Nrf2 pathway. **A** Western blotting was used to detect LC3 II/LC3 I, Beclin-1, and P62. **B** LC3 was detected by immunofluorescence. All values represent means ± SD (n = 3 biological replicates). Compared with NC group, $P < 0.001$ (***), $P < 0.0001$ (****); Compared with aconitine, $P < 0.001$ (###); Compared with NAC, $P < 0.05$ ($)

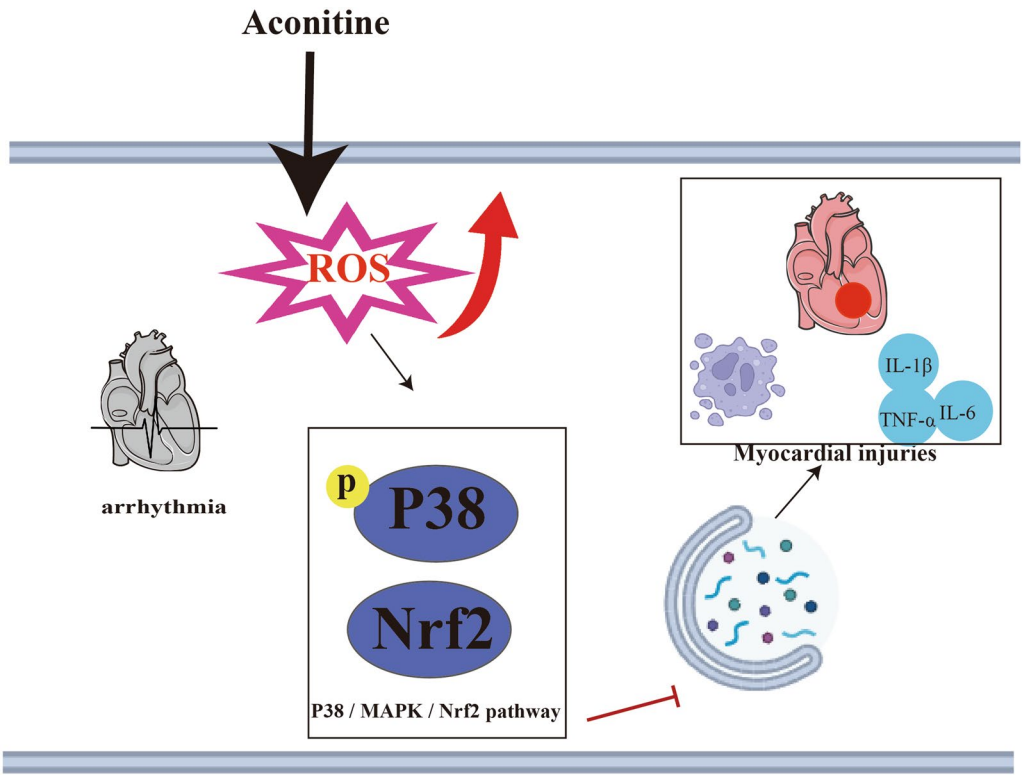

Fig. 9 Aconitine can activate the P38 / MAPK/Nrf2 pathway through ROS, promote autophagy and induce myocardial injury

that aconitine induced damage to H9c2, evidenced by decreased cell viability, increased levels of apoptotic proteins, and elevated inflammatory factors.

The p38/MAPK pathway is involved in numerous physiological and pathological processes, including apoptosis, cellular stress, cell cycle regulation, and inflammatory response [25]. This pathway can be activated by various environmental stressors and inflammatory cytokines [26]. Importantly, the p38/MAPK pathway plays a crucial role in cardiac function, particularly in myocardial ischemia/reperfusion injury, where it regulates gene expression in the heart and influences hypertrophy, inflammatory response, energy metabolism, contractile function, proliferation and apoptosis of cardiomyocytes [27]. The cardiotoxicity of aconitine has also attracted a lot of attention [28]. In this study, we investigated the relationship between aconitine and the p38/MAPK pathway in H9c2. We found that aconitine can increase the ratio of p-p38 to p38. This suggests that aconitine can activate the p38/MAPK pathway. Chu Yan yang et al. found that aconitine can activate the p38/MAPK pathway [29]. Aconitine can increase the expression of P38 [30]. The Nrf2 pathway is a key cellular defense mechanism that primarily responds to oxidative stress and exogenous or endogenous stress by regulating the expression of antioxidant and detoxification genes [31, 32]. Upregulation of the Nrf2 pathway has been shown to mitigate heart damage [33, 34]. Consistent with these findings, we observed that aconitine upregulates Nrf2 expression, further indicating that aconitine activates the P38/MAPK/Nrf2 pathway.

The generation and clearance of ROS play a positive role within cells, maintaining redox balance, facilitating redox signaling transduction, and regulating cellular functions [35]. However, large amounts of ROS are lethal to cells and surrounding tissues [36]. Previous studies have demonstrated that aconitine increases ROS levels in H9c2 cells [37]. NCA effectively reverses excessive ROS production [38] and significantly inhibits the proliferation of human liver cancer BEL-7402 cells, inducing apoptosis through the mitochondrial apoptosis pathway mediated by ROS [39]. In our study, we found that after the use of NCA, the cell viability was significantly increased, the apoptosis level was significantly decreased, and the expression levels of IL-1β, TNF-α and IL-6 were significantly decreased. Subsequently, we detected the P38/MAPK/Nrf2 pathway and found that NCA could down-regulate the expression of P38/MAPK/ Nrf2 pathway. Studies have shown that phosphorylated nuclear factor-kappa B p65 and p38 mitogen-activated protein kinase activation were blocked by NCA [40]. Our study

suggests that aconitine promotes myocardial cell damage through ROS.

Autophagy is a lysosomal dependent catabolic process that is evolutionarily conserved and includes cytoplasmic components (such as damaged organelles, protein aggregates, and lipid droplets) that are degraded and recycle their components [41]. Autophagy plays an important role in maintaining cell homeostasis in response to intracellular stress [42]. However, Inhibition of autophagy in normal cells can lead to metabolic disorders, inflammation and cancer [43]. Huang Jie et al. found that the cardiotoxic mechanism of aconitine may involve the initiation of mitochondrial dysfunction by inducing mitochondrial apoptosis and autophagy [44].In addition, some studies have suggested that the cardiotoxic mechanism of aconitine may be related to mitochondrial dysfunction caused by autophagy [44].Our study found that after inhibition of autophagy, cell viability decreased, apoptosis rate increased, apoptosis-related protein expression increased, and inflammatory factor expression increased. Fu Peng et al. also found that BNIP3-mediated mitochondrial autophagy can alleviate aconitine-induced inflammation, apoptosis and decreased cell viability [45].This suggests that aconitine inhibits autophagy. The ROS expression level was detected to increase after autophagy inhibition. This is similar to the findings of Yu Ren et al. [46]. Subsequently, we measured the expression of autophagy related proteins. The findings suggest that aconitine stimulates myocardial autophagy via the ROS-activated P38/MAPK/Nrf2 pathway.

In summary, aconitine can activate the P38 / MAPK/ Nrf2 pathway through ROS, promote autophagy and induce myocardial injury (Fig. 9).

Author contributions

Chunai Yang designed the experiments, and wrote the original manuscript. Chunai Yang, Jinxiao Fu, Fenshuang Zheng and Yangshan Fu carried out the experiment. Xueqiong Duan and Ruiling Zuo analyzed the experimental data. Chunai Yang and Jinxiao Fu participated in the supervision and revision of the paper. All authors participated in the revision of the manuscript, read and approved the final manuscript.

Funding

This work was supported by the Kunming medical joint special project-surface project (202301AY070001-213).

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest

The authors declare no competing interests.

Received: 3 June 2024 Accepted: 26 November 2024
Published online: 20 December 2024

References

1. Xiuying W, Yuanyuan L, Yi Z. Antitumor effects of aconitine in A2780 cells via estrogen receptor β-mediated apoptosis, DNA damage and migration. Mol Med Rep. 2020;22(3):2318.
2. Xiaodong L, et al. Aconitine: a potential novel treatment for systemic lupus erythematosus. J Pharmacol Sci. 2017;133(3):115.
3. Jianhua D, et al. Comparison of analgesic activities of aconitine in different mice pain models. PLoS ONE. 2021;16(4):e0249276.
4. Chan TYK. Causes and prevention of herb-induced aconite poisonings in Asia. Hum Exp Toxicol. 2011;30(12):2023.
5. Giuseppe B, et al. Accidental poisoning with Aconitum: case report and review of the literature. Clin Case Rep. 2020;8(4):696.
6. Lin CC, Chan TY, Deng JF. Clinical features and management of herb-induced aconitine poisoning. Ann Emerg Med. 2004;43(5):574.
7. Chan TY. Aconitine poisoning: a global perspective. Vet Hum Toxicol. 1994;36(4):326.
8. Deavall D, et al. Drug-induced oxidative stress and toxicity. J Toxicol. 2012;2012:645460.
9. Ichikawa Y, et al. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. J Clin Investig. 2014;124(2):617–30.
10. Ma L, et al. Sweroside alleviated aconitine-induced cardiac toxicity in H9c2 cardiomyoblast cell line. Front Pharmacol. 2018;9:1138.
11. Kong C, et al. Underlying the mechanisms of doxorubicin-induced acute cardiotoxicity: oxidative stress and cell death. Int J Biol Sci. 2022;18(2):760–70.
12. Cargnello M, Roux P. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev. 2011;75(1):50–83.
13. Shen XY, et al. Mechanisms of intermittent theta-burst stimulation attenuating nerve injury after ischemic reperfusion in rats through endoplasmic reticulum stress and ferroptosis. Mol Biol Rep. 2024;51(1):377.
14. Wang L, et al. 4-Hydroxysesamin, a modified natural compound, attenuates neuronal apoptosis after ischemic stroke via inhibiting MAPK pathway. Neuropsychiatr Dis Treat. 2024;20:523–33.
15 Kim N, et al. Cannabidiol activates MAPK pathway to induce apoptosis, paraptosis, and autophagy in colorectal cancer cells. J Cell Biochem. 2024;125:e30537.
16. Yang N, et al. Antioxidants targeting mitochondrial oxidative stress: promising neuroprotectants for epilepsy. Oxid Med Cell Longev. 2020;2020:6687185.
17. Liu J, et al. Chenodeoxycholic acid suppresses AML progression through promoting lipid peroxidation via ROS/p38 MAPK/DGAT1 pathway and inhibiting M2 macrophage polarization. Redox Biol. 2022;56:102452.
18. Wenlin W, et al. Aconitine induces autophagy via activating oxidative DNA damage-mediated AMPK/ULK1 signaling pathway in H9c2 cells. J Ethnopharmacol. 2021;282:114631.
19. Guo Y, et al. HBB contributes to individualized aconitine-induced cardiotoxicity in mice via interfering with ABHD5/AMPK/HDAC4 axis. Acta Pharmacol Sinica. 2024;45:1224.
20. Xiang G, et al. Antitumor effects and potential mechanisms of aconitine based on preclinical studies: an updated systematic review and meta-analysis. Front Pharmacol. 2023;14:1172939.
21. Kohara S, et al. Severe aconite poisoning successfully treated with veno-arterial extracorporeal membrane oxygenation: a case report. World J Clin Cases. 2024;12(2):399–404.
22. Zhang S, et al. Bupleurum exerts antiarrhythmic effects by inhibiting L-type calcium channels in mouse ventricular myocytes. Biochem Biophys Res Commun. 2024;691:149322.
23. Zhang X, et al. Aconitine induces brain tissue damage by increasing the permeability of the cerebral blood-brain barrier and over-activating endoplasmic reticulum stress. Am J Transl Res. 2022;14(5):3216–24.
24. Zhang Q, et al. Benzoylaconitine: a promising ACE2-targeted agonist for enhancing cardiac function in heart failure. Free Radical Biol Med. 2024;214:206–18.
25. Cheng Y, et al. Virus-induced p38 MAPK activation facilitates viral infection. Theranostics. 2020;10(26):12223–40.

26. He D, et al. Gut stem cell aging is driven by mTORC1 via a p38 MAPK-p53 pathway. Nat Commun. 2020;11(1):37.
27. Bottermann K, et al. Cardiomyocyte p38 MAPKα suppresses a heart-adipose tissue-neutrophil crosstalk in heart failure development. Basic Res Cardiol. 2022;117(1):48.
28. Zhou W, et al. Cardiac efficacy and toxicity of aconitine: a new frontier for the ancient poison. Med Res Rev. 2021;41(3):1798–811.
29. Yang C, et al. Aconitine induces TRPV2-mediated Ca influx through the p38 MAPK signal and promotes cardiomyocyte apoptosis. Evid-based Complement Altern Med eCAM. 2021;2021:9567056.
30. Li M, et al. Aconitine induces cardiotoxicity through regulation of calcium signaling pathway in zebrafish embryos and in H9c2 cells. J Appl Toxicol JAT. 2020;40(6):780–93.
31. Mondal G, Debnath J. NRF2 activates macropinocytosis upon autophagy inhibition. Cancer Cell. 2021;39(5):596–8.
32. Weiss-Sadan T, et al. NRF2 activation induces NADH-reductive stress, providing a metabolic vulnerability in lung cancer. Cell Metab. 2023;35(3):487-503.e7.
33. Packer M. Qiliqiangxin: a multifaceted holistic treatment for heart failure or a pharmacological probe for the identification of cardioprotective mechanisms? Eur J Heart Fail. 2023;25(12):2130–43.
34. Liu J, et al. The E3 ligase TRIM16 is a key suppressor of pathological cardiac hypertrophy. Circ Res. 2022;130(10):1586–600.
35. Lennicke C, Cochemé H. Redox metabolism: ROS as specific molecular regulators of cell signaling and function. Mol Cell. 2021;81(18):3691–707.
36. Hecht F, et al. Regulation of antioxidants in cancer. Mol Cell. 2024;84(1):23–33.
37. Wang W, et al. Aconitine induces autophagy via activating oxidative DNA damage-mediated AMPK/ULK1 signaling pathway in H9c2 cells. J Ethnopharmacol. 2022;282:114631.
38 Kang W, et al. *Peucedanum japonicum* therapeutic potential of Thunb. And its active components in a delayed corneal wound healing model following blue light irradiation-induced oxidative stress. Antioxidants (Basel, Switzerland). 2023;12(6):1171.
39. Ding Y, et al. Neochamaejasmin A induces mitochondrial-mediated apoptosis in human hepatoma cells via ROS-dependent activation of the ERK1/2/JNK signaling pathway. Oxid Med Cell Longev. 2020;2020:3237150.
40. Kim M, et al. Neochlorogenic acid inhibits lipopolysaccharide-induced activation and pro-inflammatory responses in BV2 microglial cells. Neurochem Res. 2015;40(9):1792–8.
41. Chang C, Jensen L, Hurley J. Autophagosome biogenesis comes out of the black box. Nat Cell Biol. 2021;23(5):450–6.
42. Kitada M, Koya D. Autophagy in metabolic disease and ageing. Nat Rev Endocrinol. 2021;17(11):647–61.
43. Wilson N, et al. The autophagy-NAD axis in longevity and disease. Trends Cell Biol. 2023;33(9):788–802.
44. Jiang H, et al. An updated meta-analysis based on the preclinical evidence of mechanism of aconitine-induced cardiotoxicity. Front Pharmacol. 2022;13:900842.
45. Peng F, et al. Aconitine induces cardiomyocyte damage by mitigating BNIP3-dependent mitophagy and the TNFα-NLRP3 signalling axis. Cell Prolif. 2020;53(1):e12701.
46. Ren Y, et al. Exosomes from adipose-derived stem cells alleviate premature ovarian failure via blockage of autophagy and AMPK/mTOR pathway. PeerJ. 2023;11:e16517.

Publisher's Note