Institute of Pharmacology[1], School of Basic Medical Science, Lanzhou University, Key Laboratory of Preclinical Study for New Drug of Gansu Province; Gansu Provincial Cancer Hospital[2]; Gansu Provincial Hospital[3], Lanzhou, China

Ampelopsin sodium induces mitochondrial-mediated apoptosis in human lung adenocarcinoma SPC-A-1 cell line

Jun-Feng Jiang[1,2], Jing Zhai[1,3], Zhou-Ru-Jun Liu[1], Li Chao[1], Yun-Feng Zhao[1], Yong-Jie Wu[1], Ming-Xia Cui[1]

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Ming-Xia Cui, Yong-Jie Wu, Department of Pharmacology, School of Basic Medical Sciences, Lanzhou University, No 199, Dongang Xi Road, Lanzhou 730000, China
cuimx@lzu.edu.cn; wuyj@lzu.edu.cn

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Ampelopsin is a well-known flavonoid which has variety of biological and pharmacological actions including anticancer effects and induction of apoptosis on the several cancer cell lines. The present study aimed to evaluate the role of ampelopsin sodium (Amp-Na) in the mitochondrial-mediated apoptosis of human lung adenocarcinoma SPC-A-1 cells. The analysis of cell proliferation and ultrastructure were performed. Furthermore, to clarify its action mechanism by determining the mitochondrial membrane potential (ΔΨm), intracellular calcium (Ca^{2+}) concentration, mitochondrial nitric oxide (NO) level and total ATPase activity. The results showed that Amp-Na markedly inhibited the SPC-A-1 cell proliferation and caused ultrastructural apoptosis feature in SPC-A-1 cells in a dose-dependent manner. Amp-Na led to a rapid and sustained Ca^{2+} elevation and ΔΨm reduction, and induced the mitochondrial NO production and decreased the total ATPase activity in SPC-A-1 cells. The results enhance the potential of Amp-Na as a therapeutic drug for treating lung cancer, and provide new information for mechanism of Amp-Na which induces mitochondrial-mediated apoptosis in tumor cells.

1. Introduction

Lung cancer is the first deadliest types of cancer worldwide (Jemal et al. 2002). Non-small cell lung cancer (NSCLC) accounts for approximately 80% of all lung cancers and is one of the leading causes of death in all lung cancer patients due to its metastatic spread. Current standard for locally advanced NSCLC is based on the combination of platinum compounds and a third-generation cytotoxic agent (Einhorn 2008). High efficiency and low toxicity chemotherapeutic candidates for NSCLC are therefore urgently needed to explore.

Ampelopsin (3,5,7,39,49,59-hexahydroxy-2,3-dihydroflavonol, AMP) is a small flavonoid molecule and the major bioactive component of *Ampelopsis grossedentata* which is widely distributed in South China. AMP has received increased attention in the last few years, because it's wide variety of biological and pharmacological actions, including anti-inflammatory, anti-hypertension, antioxidant, hepatoprotective, and anticancer (Zhang et al. 2012; Ni et al. 2012) effects. AMP has the ability to induce apoptosis in tumor cell lines (Lu et al. 2015; Qi et al. 2015; Chen et al. 2015; Zhou et al. 2014), but AMP induced tumor cell apoptosis through the mitochondrial-mediated pathway has not been reported yet. In addition, AMP is insoluble and unstable with a short plasma half-life and unsuitable for clinical use. To improve the deficiencies, AMP is prepared as a sodium salt [ampelopsin sodium (Amp-Na)]. The present study aimed to evaluate the role of Amp-Na in the mitochondrial-mediated apoptosis of SPC-A-1 cells.

2. Investigations and results

2.1. Inhibitory effects of Amp-Na on SPC-A-1 cell proliferation

As shown in Fig. 1, the proliferation of SPC-A-1 cells was significantly inhibited by Amp-Na 6.25-200 $\mu g \cdot ml^{-1}$ and carboplatin (CBP) 3.13-100 $\mu g \cdot ml^{-1}$ in a concentration-dependent manner after 48 h of treatment. The IC_{50} values were 48.3 and 18.6 $\mu g \cdot ml^{-1}$, respectively.

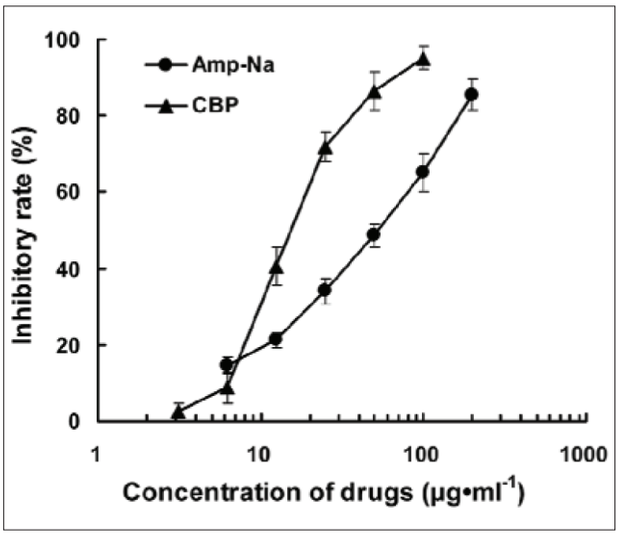
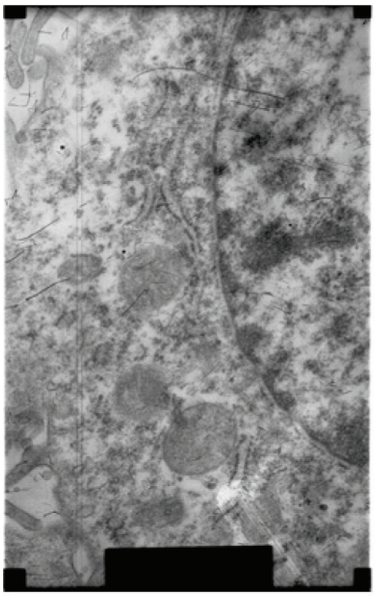


Fig. 1: Inhibitory effects on SPC-A-1 cell proliferation after treatment with Amp-Na or carboplatin (CBP) for 48 hours. $n = 3$

2.2. Damage effects of Amp-Na on SPC-A-1 cell ultrastructure

The ultrastructural alterations of SPC-A-1 cells were investigated by transmission electron microscope (TEM) 48 h after Amp-Na treatment. Micrographs show mitochondrial swelling, reduced microvilli, and vacuoles in cytoplasm. In the high dose group of Amp-Na, mitochondrial crista ruptured and even disappeared, and the nuclear envelope break down. Results are shown in Fig. 2.



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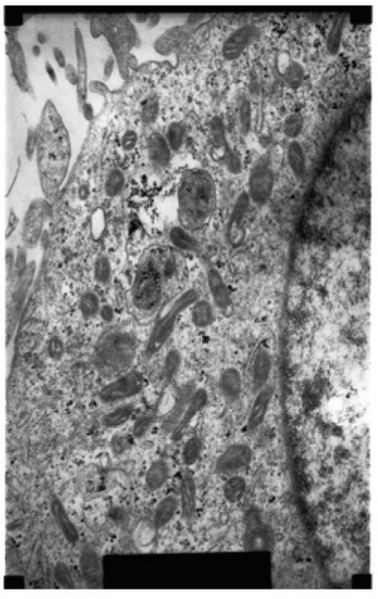
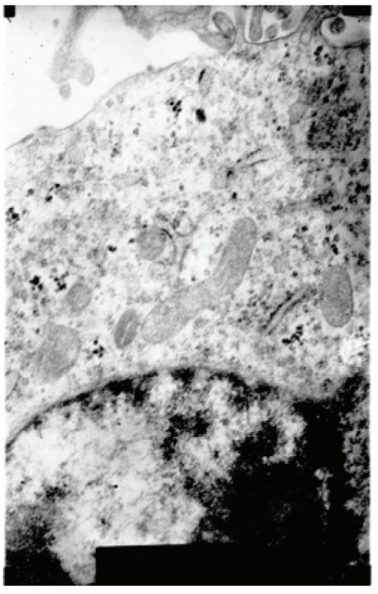
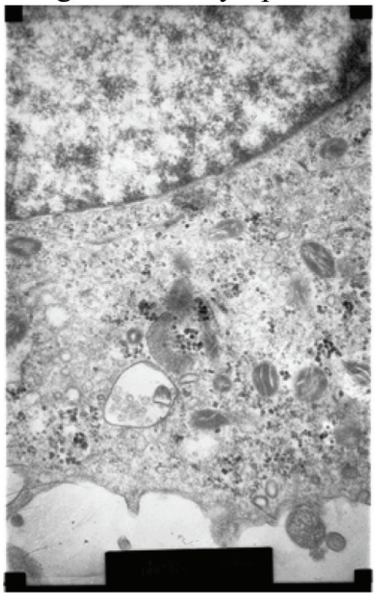
Micrograph shows integral and clear karyotheca, rich microvilli and normal organelles in cytoplasm.



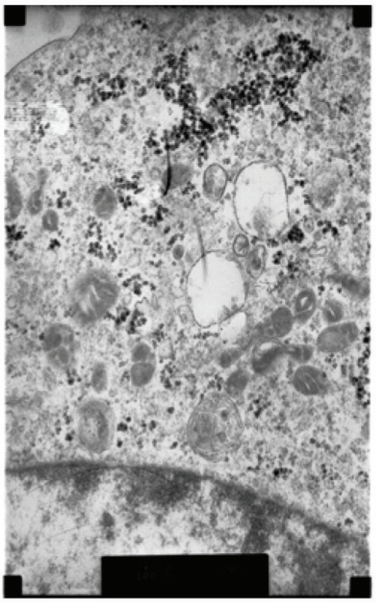
CBP 25 μg·ml^{-1}
Carboplatin (CBP) induced mitochondrial swelling and increased the number of mitochondria.



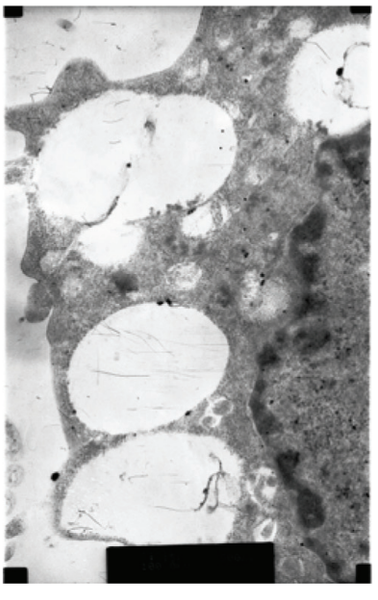
Amp-Na 25 μg·ml^{-1}
Micrograph shows mitochondrial mild swelling



Amp-Na 50 μg·ml^{-1}
The nuclear membrane was still intact. But there were mitochondrial obvious swelling and vacuole-like structures in the cytoplasm



Amp-Na 100 μg·ml^{-1}
Micrograph shows reduced microvilli, obvious mitochondrial swelling, and vacuoles in cytoplasm.



Amp-Na 200 μg·ml^{-1}
High concentration of Amp-Na induced mitochondrial crista disappeared, and nuclear envelope breakdown.

Fig. 2: Ultrastructural alterations of apoptosis investigated by transmission electron microscope (TEM) 48 hours after Amp-Na treatment in SPC-A-1 cells (× 20000)

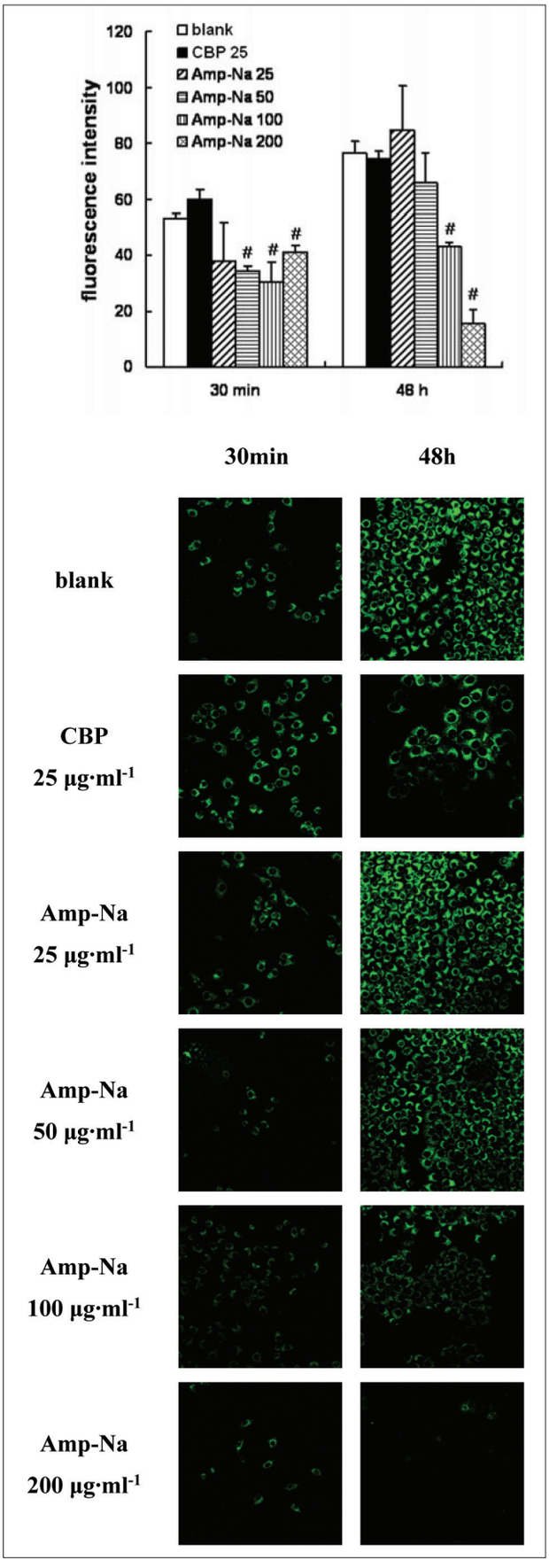


Fig. 3: The mitochondrial membrane potential (ΔΨm) of SPC-A-1 cells after Amp-Na treatment for 30 min and 48 hours. All data are mean ± S.D., $n = 3$. # $P < 0.01$ versus blank group.

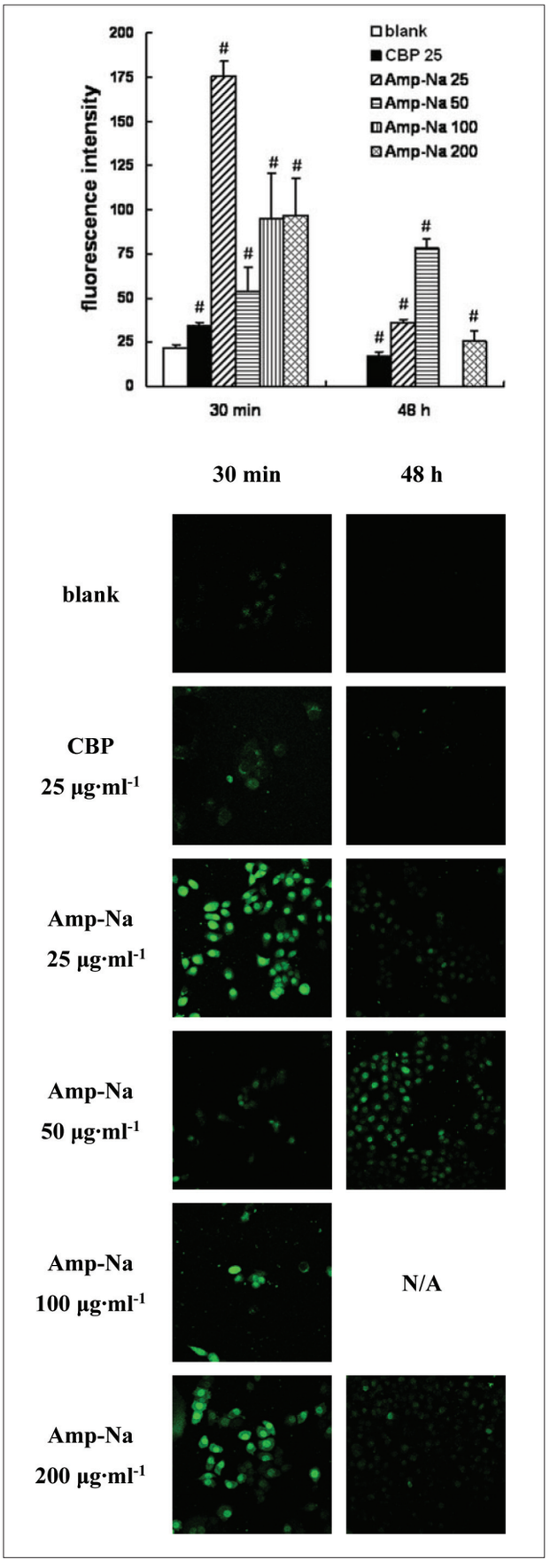


Fig. 4: The intracellular calcium level of SPC-A-1 cells after Amp-Na treatment for 30 min and 48 hours. All data are mean ± S.D., $n = 3$. # $P < 0.01$ versus blank group.

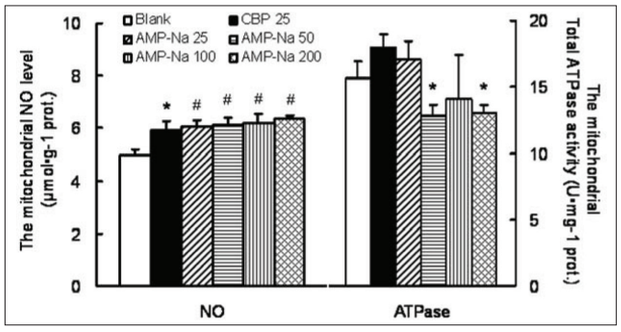


Fig. 5: The mitochondrial NO level and total ATPase activity of SPC-A-1 cells after Amp-Na treatment for 48 hours. All data are mean ± S.D., $n = 3$. *$P < 0.05$, # $P < 0.01$ versus blank group.

2.3. AMP-Na reduced mitochondrial membrane potential ($\Delta\Psi m$)

As shown in Fig. 3, the mitochondrial membrane potential (ΔΨm) of SPC-A-1 cells was significantly decreased ($P < 0.01$) 30 min after Amp-Na 50, 100, and 200 μg·ml^{-1} treatment, and 48 h after Amp-Na 100 and 200 μg·ml^{-1} treatment. CBP 25 μg·ml^{-1} had no effect on ΔΨm.

2.4. AMP-Na increased intracellular calcium concentration

The intracellular calcium concentration was both significantly increased ($P < 0.01$) in Amp-Na 25-200 μg·ml^{-1} and CBP 25 μg·ml^{-1} groups after treatment for 30 min and 48 h (Fig. 4).

2.5. AMP-Na increased nitric oxide (NO) level and decreased total ATPase activity in mitochondria

After treatment for 48 h, the mitochondrial NO level in SPC-A-1 cells was obviously increased in Amp-Na 25-200 μg·ml^{-1} groups in a concentration-dependent manner ($r^2 = 0.986$). The mitochondrial total ATPase activity was significantly decreased by Amp-Na 50 and 200 μg·ml^{-1}. CBP 25 μg·ml^{-1} also significantly reduced the mitochondrial NO level but had no effect on total ATPase activity (Fig. 5).

3. Discussion

Cancer cells lose the ability of spontaneous apoptosis which is one of the important reasons of the occurrence and development of tumors. A highly efficient and little toxic antitumor drug should not only inhibit cell proliferation, but also induce apoptosis of tumor cells (Ferreira et a1. 2000).

Mitochondria, the ATP production center, play a very important role in the processes of apoptosis, aging, cancer and signal transduction, and are seen as a key element of apoptosis (Green and Reed 1998). The reduction of ΔΨm is considered to be one of the earliest events in the apoptotic reaction cascade, which occurred before the appearance of nuclear apoptosis features. As an important member of the intracellular signaling pathways and early signal of cell apoptosis, Ca^{2+} effectively induces mitochondrial membrane permeabilization and is significantly and sustained increased before apoptosis (Giacomello et al, 2007). The results of the present study suggest that intracellular Ca^{2+} elevation may induce the opening of mitochondrial permeability transition pores (MPTP) and the loss of ΔΨm, lead to mitochondrial swelling, and then trigger the mitochondrial-induced apoptotic pathway.

Nitric oxide (NO) has gained much attention in the past decades in several research fields due to its properties as a free radical and signaling molecule in different cellular pathways including apoptosis (Shimaoka et al. 1995; Sumitani et al. 1997; Nisoli et al. 2003). NO as a ubiquitous signaling molecule has a regulatory role in the occurrence and development of tumors (Forstermann et al. 2012; Carradori et al. 2015). Our results show that the increased mitochondrial NO level induced by Amp-Na may be one mechanism contributing to apoptosis in SPC-A-1 cells.

ATPase plays an important role in the maintenance of cell permeability and energy transformation in the biological system. The results suggest that the decrease of mitochondrial total ATPase activity is related to mitochondrial damage after Amp-Na treatment in SPC-A-1 cells.

In summary, our results indicate that Amp-Na can induce early events in the apoptotic cascade, such as rapid and sustained Ca^{2+} elevation, loss of ΔΨm, an increase of mitochondrial NO level and decrease of total ATPase activity in SPC-A-1 cells. The results from this study support the potential of Amp-Na as a therapeutic drug for treating lung cancer, and provide new information about the mechanism by which Amp-Na induces mitochondria-mediated apoptosis in tumor cells.

4. Experimental

4.1. Drug

Ampelopsin sodium (Amp-Na, the purity is 98.8%) and its special diluents (0.1 mol·L^{-1} phosphate buffer, pH 6.8) were manufactured by the Guangdong Taihe Pharmaceutical Co., Ltd. (Guangzhou, China). Carboplatin (CBP) was manufactured by the Qilu Pharmaceutical Co., Ltd. (Jinan, China).

4.2. Cell line

Human lung adenocarcinoma cell line SPC-A-1 was purchased from Shanghai Institutes for Biological sciences (Shanghai, China). SPC-A-1 cells were cultured in RPMI-1640 medium (GIBCO, Carlsbad, California, USA) supplemented with 10% heat inactivated fetal bovine serum, 1×10^5 IU·L^{-1} penicillin sodium, and 100 mg·L^{-1} streptomycin sulfate, in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

4.3. Experimental protocol

SPC-A-1 cells were treated with drugs for 24 h or 48 h after 24 h cultured. The analysis of cell proliferation, cell ultrastructure, mitochondrial membrane potential (ΔΨm), intracellular calcium concentration, mitochondrial NO level and total ATPase activity were performed. The effect of Amp-Na on SPC-A-1 cell proliferation was evaluated by microculture tetrazolium (MTT) assay (Guo et al. 2015). The changes of cell ultrastructure were observed by Transmission electron microscope (JEN-100CX, Japan). The levels of mitochondrial membrane potential (ΔΨm) and intracellular calcium concentration was monitored using Rhodamine-123 (Sigma, USA) and Fluo-3/AM Ester (Biotium, USA) fluorescence assay, respectively, by laser scanning confocal microscopy (Leica TCSSP2, Leica Microsystems Heidelberg GmbH, Mannheim, Germany). Mitochondrial NO levels and total ATPase activities were measured in accordance with the kit manual (Kits were products of Jiancheng Bioengineering Institute, Nanjing, China).

4.4. Statistical analysis

The data were expressed as mean±S.D. Statistical comparisons between groups were carried out using ANOVA followed by Tukey's *post hoc* test. $P < 0.05$ was considered significant.

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