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# The inhibiting effects of Laggera alata flavone on human ovarian cancer HO-8910 cells proliferation and its mechanism in vitro

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**Abstract** ***Objective:*** The purpose of this study was to investigate the effect of Laggera alata flavonen (LAF) on the inhibiting effect of human ovarian cancer HO-8910 cells proliferation and its possible mechanism *in vitro*. ***Methods:*** Human ovarian cancer HO-8910 cells were cultured *in vitro*. Inhibitory effect of LAF on the viability of HO-8910 cells was evaluated by the MTT assay. Apoptotic effect of different concentrations of LAF on HO-8910 cells was assessed by AO/EB staining and FCM with propidium iodide (PI) staining. Expression of proteins related to apoptosis was analyzed by Western blot. ***Results:*** LAF significantly inhibited the viability of HO-8910 cells proliferation in a dose-dependent and time-dependent manner, there were statistical significance compared with NS group ($P < 0.05$), and the $IC_{50}$ was 4.28 μg/mL for 48 h. The cells treated with LAF showed typical morphological change and apoptotic rate increased by FCM in a dose-dependent, and there was notable difference compared with NS group ($P < 0.05$). Western blot showed that expression of Fas, caspase-8, tBid and Cyto-c proteins were up-regulated after treatment with LAF for 48 h in a concentration dependent. ***Conclusion:*** LAF could inhibit HO-8910 cells proliferation and induce apoptosis, which may be through the pathway of death receptor *in vitro*.

**Key words** ovarian cancer; Laggera alata flavonen (LAF); apoptosis

Laggera alata, as a medical plant, posses potent anti-inflammatory, anti-bacterial, anti-febrile, anti-tumor, and alexipharmic actions and so on, and it has been used in folk and Chinese medicine for a few hundred years, and the plant widely distribute in China [1]. Laggera alata flavonen (LAF) is extract from laggera alata, nowadays, it has been reported possessing anti-inflammatory, anti-virus, and so on, but its anti-tumor effect was not reported [2]. In this present study, experiments were designed to investigate the inhibiting effects of LAF on HO-8910 cells proliferation, induction of apoptosis and its related possible biological mechanism *in vitro*.

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## Materials and methods

### Materials

LAF was extracted, isolated, and purified in the Department of Pharmacology of Guangzhou Medical University. Human ovarian cancer HO-8910 cells were purchased from the China Center for Type Culture Collection. RPMI-1640 medium and fetal calf serum, FCS) was tained from Gibco Company (USA), and DMSO was purchased Company Amresco (USA). The 3-(4,5-dimethythiazol-2-yl)-2,5-iphenylterazoliumromide (MTT) was bought from Sigma Company (USA). DDP was purchased from Hengrui Medicine Co., Ltd (China). AO/EB staining kit, mouse anti-Fas, anti-cyt-c, anti-truncated binding interface database (tBid) monoclonal antibody, rabbi anti-caspase-8 monoclonal antibody were obtained from Beyotime Company (China).

### Cell culture

Human ovarian cancer HO-8910 cells were maintained in RPMI-1640 medium supplemented with 10% FCS at 37 ℃ in a humidified atmosphere of 5% $CO_2$. Exponentially growing cells were used in experiments [3].

### Cell proliferation inhibition analysis

The effect of different concentration of LAF (1, 3, and 10 μg/mL) on the proliferation ability of HO-8910 cells was determined by using MTT assy. The $2.5 \times 10^4$ cells/mL exponentially growing cells were plated into 96-well flat-bottom micro-plates, and treated with NS, 0.2% DMSO, DDP 0.1 μg/mL, LAF (1, 3, and 10 μg/mL). The total incubational volume of each well was 200 μL. After incubation for 24, 48, and 72 h, 20 μL MTT was added to each well and incubated for additional 6 h, and then the supernatants were removed and 100 μL. DMSO was added to dissolve the fromazan crystals. The viable cell number was directly proportional to the production of formazan. The plate was then read in a micro-plate reader (ELX-800) at 570 nm. We counted the relative viable cells inhibit rate (IR), $IR = (1 - A_{\text{the average value of LAF}} / A_{\text{the average value of NS}}) \times 100\%$ [4]. The experiment was repeated three times.

### AO/EB staining assay

The effect of LAF on morphological changes of HO-8910 cells apoptosis was determined by using AO/EB staining assay. 900 μL ($5 \times 10^3$ cells/mL ) exponentially growing cells were plated into 24-well flat-bottom micro-plates, after incubation for 24 h, 100 μL of different experimental factors was added to each well and incubated for additional 48 h and the final concentration were NS, 0.2% DMSO, DDP 0.1 μg/mL and LAF 1, 3, and 10 μg/mL). And then removed the culture medium and stained in AO/EB. At last we used 340 nm wavelength ultraviolet excitation florescene observed under microscope, and camera [5].

### Cell apoptosis analysis

The effect of LAF on apoptotic rate of HO-8910 cells was determined by using FCM assay. Exponentially growing cells were simultaneously dealt in RPMI-1640 medium supplemented with 1% FCS for 24 h, and then the cells were exposed in different experimental drug groups and continued to incubate in RPMI-1640 medium supplemented with 10% FCS for 48 h. Then the cells were harvested and washed by cold PBS twice and fixed in ethanol 70% (4 ℃), then stained in propidium iodide (PI) in darkness. Distribution of cell cycle was analyzed by flow cytometry [6]. The experiment was repeated three times.

### Western blot analysis

HO-8910 cells were exposed to NS, 0.2% DMSO, DDP 0.1 μg/mL and LAF (1, 3, and 10 μg/mL) for 48 h, and then washed 3 times in cold PBS, then harvested and treated with cell lysate. The protein amount was detected with BCA kit, samples containing 30 μg of proteins were separated by 10% SDS-PAGE gel electrophoresis, and then electro-transferred to the PVDF membranes. Membranes were blocked with TBST containing 5% non-flat dry milk and incubated with the indicated primary antibodies overnight at 4 ℃, and then membranes were incubated with HRP-conjugated second antibody. Protein-antibodies complexes were detected by enhanced chemiluminescene according to the manufacturer's recommendations. Band densities in western blot measured using Imaging J for windows software [7]. The experiment was repeated three times.

### Statistical analysis

All the experimental data were expressed in $\bar{\chi} \pm s$, and statistic analyses were performed using SPSS 15.0 software. Differences between groups were examined with One-Way ANOVA, and a probability level of 0.05 was chosen for statistical significance.

## Results

### Effect of LAF on the inhibiting proliferation of HO-8910 cells

MTT assay showed that LAF significantly inhibited the viability of human ovarian cancer HO-8910 cells in a dose-dependent and time-dependent manner. There were statistical significance when cells exposed to the different concentration of LAF (1, 3, and 10 μg/mL) for 24, 48, and 72 h compared with NS group ($P < 0.05$), and there were significantly difference between any two different concentration of LAF experimental groups ($P < 0.05$), and the $IC_{50}$ was 4.28 μg/mL for 48 h. While the inhibiting effect of DDP 0.1 μg/mL group was approximately equal to LAF 3 μg/mL ($P > 0.05$; Fig. 1).

### Effect of LAF on the apoptotic morphological changes of HO-8910 cells

AO/EB staining assay showed there were not apoptotic morphological changes when HO-8910 cells were exposed to NS group, 0.2% DMSO group for 48 h. While cells were treated with LAF (1, 3, and 10 μg/mL) for 48 h, there were typical apoptotic morphological changes in dose-dependant, such as nuclear condensation, nuclear fragmentation and so on, the effect of LAF 3 μg/mL was similar to DDP 0.1 μg/mL group (Fig. 2).

### Effect of LAF on the apoptotic rate of HO-8910 cells

FCM with PI staining showed apoptosis of HO-8910 cells increased in dose-dependant when they exposed in different concentrations of LAF (1, 3, and 10 μg/mL) for

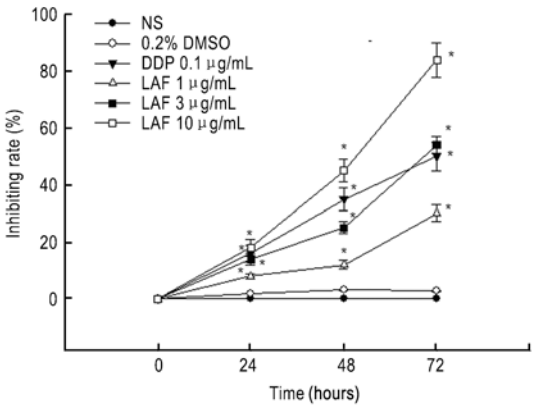


**Fig. 1** The inhibiting effect of LAF with different concentrations for different time on HO-8910 cells proliferation. * $P < 0.05$ vs NS group

48 h, the LAF groups superior to the NS groups in the apoptotic rate ($P < 0.05$). There was no statistical difference between NS groups and 0.2% DMSO group. There were significantly difference among different groups of LAF in the apoptotic rate ($P < 0.05$). DDP group superior to NS group in the apoptotic rate ($P < 0.05$), and the apoptotic rate of DDP 0.1 μg/mL was similar to the rate of LAF 3 μg/mL ($P > 0.05$; Fig. 3).

### Effect of LAF on the expression of proteins HO-8910 cells related to the pathway of death receptor

Western blot assay showed the expressions of Fas, Caspase-8, tBid and Cyto-c proteins were up-regulated in dose-dependant manner when HO-8910 cells exposed to different concentration of LAF (1, 3, and 10 μg/mL) for 48 h compared with NS groups ($P < 0.05$), which showed LAF inhibited HO-8910 cells proliferation and induced apoptosis might be through the pathway of death receptor *in vitro* (Fig. 4).

## Discussion

Human ovarian cancer is one of common malignant tumors in human, its occurrence and development are not ease to perceive in clinic, and nowadays the occurrence of ovarian cancer tends to younger staff, which seriously threaten human's life quality [8]. Chemotherapy is one of effective treatment to ovarian cancer in clinic, and the new generation of chemotherapy medicine and the application of neoadjuvant chemotherapy improve ovarian cancer survival rate and life quality, so it is prime task to develop new chemotherapy medicine to cure ovarian cancer [9].

Apoptosis is a manifestation of individual cell of programmed death *in vivo*, it determined by factors outside the body which trigger cell death program stored within cells and lead cells to death, apoptosis play an important role in the occurrence and development of embryo, cell transition from the old to mature, hormone-dependent physiological degeneration, atrophy and aging as well as autoimmune diseases and tumor development and so on [10]. LAF is extract from Laggera alata that is used traditional herbal plant curing inflammatory, febrile, alexipharmic drug, tumors and so on, and it has been reported possessing anti-inflammatory, anti-virus, and so on, but its anti-tumor effect was not reported [11]. In this present study, we found that LAF could significantly inhibit human ovarian cancer HO-8910 cells proliferation and induce them apoptosis, which suggested LAF is a proper

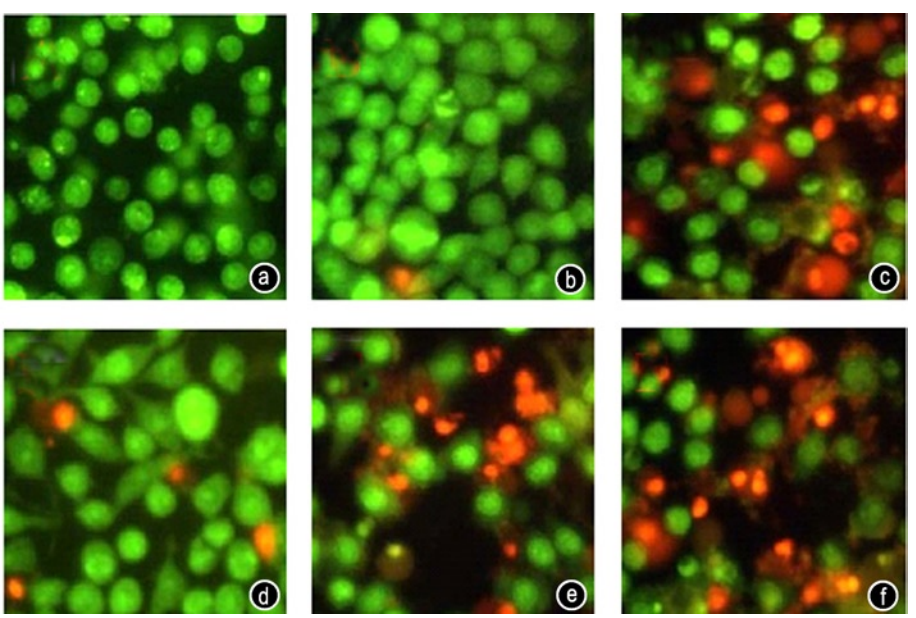


**Fig. 2** The effect of LAF on the apoptotic morphological changes of HO-8910 cells. (a) NS group; (b) 0.2% DMSO group; (c) DDP 0.1 μg/mL group; (d) LAF 1 μg/mL group; (e) LAF 3 μg/mL group; (f) LAF 10 μg/mL group

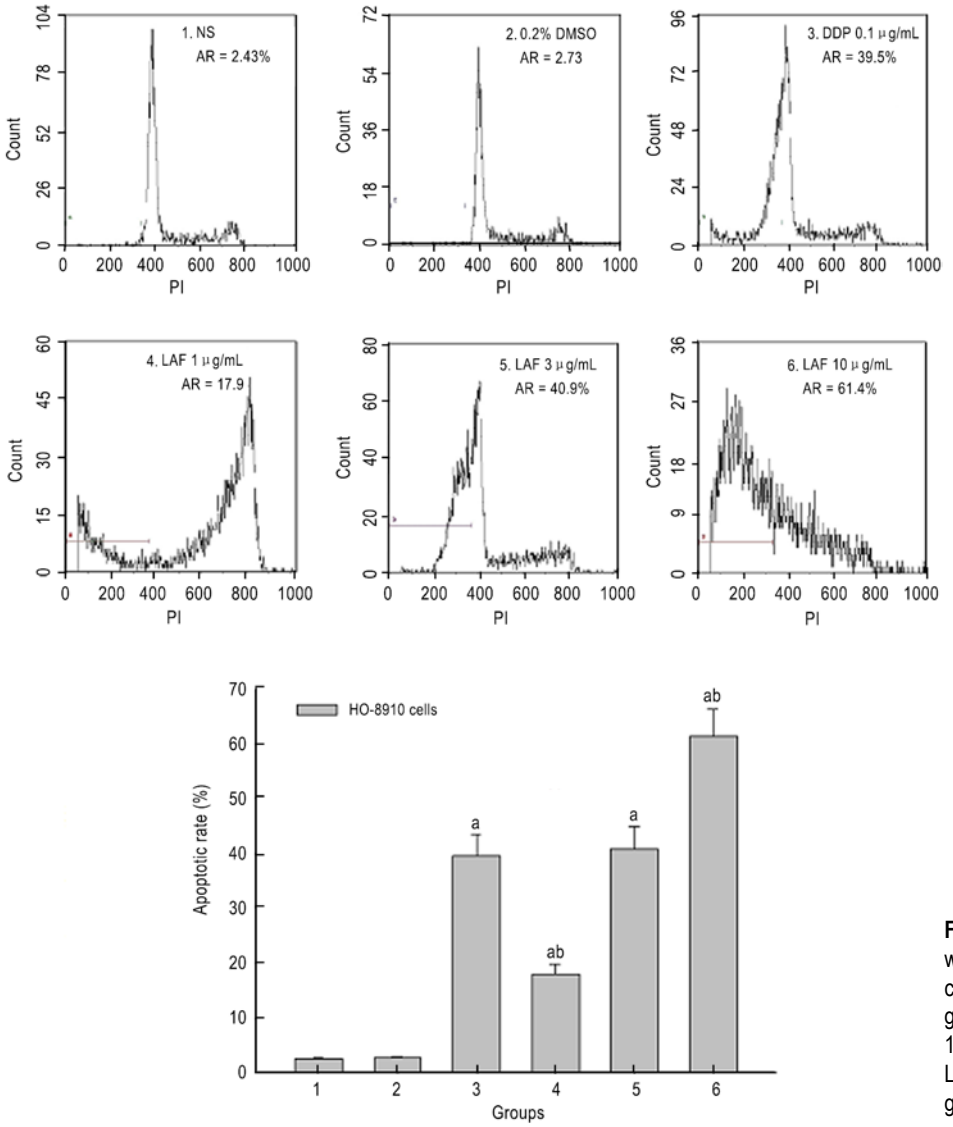


**Fig. 3** The apoptotic induction of LAF with different concentrations on HO-8910 cells for 48 h. 1: NS group; 2: 0.2% DMSO group; 3: DDP 0.1 μg/mL group; 4: LAF 1 μg/mL group; 5: LAF 3 μg/mL group; 6: LAF 10 μg/mL group. [a] $P < 0.05$ vs NS group; [b] $P < 0.01$ vs NS group

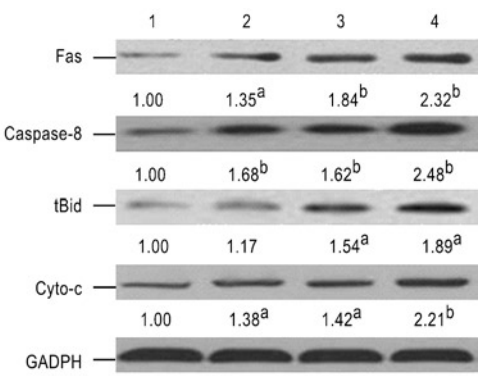


**Fig. 4** The expression of proteins of HO-8910 cells treated with different concentrations of LAF for 48 h. (1) NS group; (2) LAF 1 μg/mL group; (3) LAF 3 μg/mL group; (4) LAF 10 μg/mL group. [a] $P < 0.05$ vs NS group; [b] $P < 0.01$ vs NS group

candidate to therapy human ovarian tumors.

The apoptotic pathway of death receptor mediation is one of typical dead ways of cells, in this dead way, dead ligant, such as TNF-α, Fas L, can combine with Fas receptor which lies in cell membrane [12], and the activated Fas owning the domain that possess the special function of collecting adpter proteins with function combining procaspase-8 to construct a new complex called Fas L-Fas-FADD-procaspase-8, and in this complex, the high dose of procaspase-8 can be self-activated, and then caspase-8 were released into cell cytoplasm, and trigger the caspase cascade reaction and induce apoptosis [13–14]. Simultaneously caspase-8 can activate the protein of Bid which can form tBid that possess capacity to damage mitochodria and cause Cyto-c release and trigger apoptosis [15]. Our study showed the proliferation of HO-8910 cells were inhibited and apoptosis were induced when cells exposed to dif-

ferent concentration of LAF (1, 3, and 10 μg/mL). Meanwhile, the expression of Fas, Caspase-8, tBid, and Cyto-c proteins were up-regulated in a dose-dependent of LAF, which suggest the inhibiting effect of proliferation and the inducing action of apoptosis of ovarian cancer HO-8910 cells were induced by LAF through the apoptotic pathway of death receptor mediation *in vitro*.

In summary, LAF could inhibit the proliferation of human ovarian cancer HO-8910 cells and induce apoptosis through the death receptor pathway, in this study we observed the inhibiting effect of proliferation and the inducing action of apoptosis HO-8910 cells that LAF induced and its possible mechanism, which show LAF has great value to develop as a new kind of chemotherapy medicine. However, the current study is also limited *in vitro*, and the *in vivo* study of related to the antitumor action of LAF needs further to perform *in vivo*.

### Conflicts of interest

The authors indicated no potential conflicts of interest.

## References


1. Hao BJ, Wu YH, Wang JG, *et al*. Hepatoprotective and antiviral properties of isochlorogenic acid A from Laggera alata against hepatitis B virus infection. J Ethnopharmacol, 2012, 144: 190–194.
2. Wu YH, Hao BJ, Shen E, *et al*. Protective properties of Laggera alata extract and its principle components against d-Galactosamine-injured hepatocytes. Sci Pharm, 2012, 80:447–456.
3. Bai J, Tan GH, Chen L, *et al.* Casticin combination with Cisplatin in sub-toxicconcentration induced apoptosis of human ovarian cancer HO-8910 cells in vitro. Chinese-German J Clin Oncol, 2013, 12: 35–39.
4. Porcheri C, Suter U, Jessberger S. Dissecting integrin-dependent regulation of neural stem cell proliferation in the adult brain. J Neurosci, 2014, 34: 5222–5232.
5. Senthilkumar N, Kurinjimalar C, Thangam R, *et al.* Further studies and biological activities of macromolecular protein R-Phycoerythrin from Portieria hornemannii. Int J Biol Macromol, 2013, 62:107–116.
6. Miao Y, Sun Q, Wen Q, *et al.* Cytotoxic effects of betaxolol on healthy corneal endothelial cells both in vitro and in vivo. Int J Ophthalmol, 2014, 7: 14-21.
7. Karim S, Mirza Z, Naseer MI, *et al.* Clinicopathological characteristics and chronology of p53 expression in the development of gastric cancer. Hepatogastroenterology, 2013, 60: 2113–2118.
8. Tuppurainen L, Sallinen H, Kokki E, *et al*. Preclinical safety, toxicology, and biodistribution study of adenoviral gene therapy with sVEGFR-2 and sVEGFR-3 combined with chemotherapy for ovarian cancer. Hum Gene Ther Clin Dev, 2013, 24: 29–37.
9. Tai WP, Hu PJ, Wu J, *et al.* The inhibition of Wnt/β-catenin signaling pathway in human colon cancer cells by sulindac. Tumori, 2014, 100: 97–101.
10. Song J, Hur BE, Bokara KK, *et al.* Agmatine improves cognitive dysfunction and prevents cell death in a streptozotocin-induced Alzheimer rat model. Yonsei Med J, 2014, 55: 689–699.
11. Matasyoh JC, Dittrich B, Schueffler A, *et al.* Larvicidal activity of metabolites from the endophytic Podospora sp. against the malaria vector Anopheles gambiae. Parasitol Res, 2011, 108: 561–566.
12. Wu MF, Yang J, Xiang T, *et al.* miR-21 targets Fas ligand-mediated apoptosis in breast cancer cell line MCF-7. J Huazhong Univ Sci Technolog Med Sci, 2014, 34: 190–194.
13. Jung EB, Lee CS. Baicalein attenuates proteasome inhibition-induced apoptosis by suppressing the activation of the mitochondrial pathway and the caspase-8- and Bid-dependent pathways. Eur J Pharmacol, 2014, 730: 116–124.
14. Raemy E, Martinou JC. Involvement of cardiolipin in tBID-induced activation of BAX during apoptosis. Chem Phys Lipids, 2014, 179: 70–74.
15. Faizi M, Salimi A, Rasoulzadeh M, *et al.* Schizophrenia induces oxidative stress and cytochrome C release in isolated rat brain mitochondria: a possible pathway for induction of apoptosis and neurodegeneration. Iran J Pharm Res, 2014, 13:93–100.


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