



Magnoflorine from *Coptis chinese* has the potential to treat DNCB-induced Atopic dermatitis by inhibiting apoptosis of keratinocyte

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ABSTRACT

Aims: In Sheng Nong's herbal classic in China, *Rhizoma coptidis*^a(RC) could be used to treat *Atopic dermatitis*^b(AD), but its core ingredient(s) and mechanism remains unknown. The present study aimed to find out the ingredients against AD and expound its mechanisms.

Materials and methods: Seven alkaloids were isolated from RC to compare the inhibition against HaCaT cells by MTT assays and apoptosis of cells stimulated with TNF- α /IFN- γ by flow cytometry. The effects of target alkaloids against AD were evaluated on DNCB^c (2,4-dinitrochlorobenzene)-induced atopic dermatitis in mice.

Key findings: Seven alkaloids were isolated from RC successfully. The results from MTT and flow cytometry indicated that among these alkaloids, only magnoflorine^d(MAG) had no obvious toxicity on cells, but could inhibit the apoptosis of the cells stimulated with TNF- α /IFN- γ . Further animal experiments confirmed that MAG significantly attenuated the AD-like symptom and inhibited the AD-induced increases in IgE/IL-4, as compared with control ($P < 0.01$). Moreover, MAG reduced the low $\Delta\psi_m$ ^e(mitochondrial membrane potential) in HaCaT cells. The results of western blotting proved that MAG inhibited apoptosis of keratinocytes through decreasing the expressions of CTSB^f (cathepsin B), Cyte C^g (cytochrome C), Bid and caspase-3/7/8/9.

Significance: Overall, MAG inhibited apoptosis by decreasing the expression of apoptotic pathway-related proteins, and laid a foundation for the study of AD mechanisms.

1. Introduction

Atopic dermatitis (AD), known as *atopic eczema*, as a chronic skin disease inflammatory.^{1,2} Eczematous disorders account for a large proportion in all skin disease, and constitute a major health problem worldwide.³ It is clinically distinguished by pruritus, dryness, erythema and eczema skin lesions.^{4,5} Currently, the treatment of AD focuses on improving the skin barrier integrity.⁴ Clinical use of antihistamines,⁶ corticosteroids or immunological agents to treat atopic dermatitis, but the side effects are also more obvious and not suitable for long term use.⁷ From the current research report on AD, it reveals that AD is

accompanying apoptosis of keratinocytes.^{8–10} Lysosomal cathepsins can be widely involved in the apoptosis of many types of cells, CTSB was found to be involved in apoptosis and caused caspase cascade to expand apoptosis.¹¹ Research indicates that Bid was an important molecule of lysosome-mediated apoptosis and could trigger the release of Cyte C from mitochondria.¹² Falling in $\Delta\psi_m$ and release of Cyte C will also induce apoptosis in general. The findings of Schwarz et al. indicate Fas-mediated apoptosis of keratinocytes is the key event in AD, keratinocytes apoptosis represents a major mechanism in the pathogenesis of AD as well as other eczematous disorders.¹³ Therefore pharmacologic inhibitors of apoptosis should be of benefit. Besides, HaCaT cells are a

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^a RC: *Rhizoma coptidis*

^b AD: *Atopic dermatitis*

^c DNCB: 2,4-dinitrochlorobenzene

^d MAG: magnoflorine

^e $\Delta\psi_m$: mitochondrial membrane potential

^f CTSB: cathepsin B

^g Cyte C: cytochrome C

human keratinocyte cell line that has been widely used as an in vitro model of epidermis.¹⁴ Incubation of HaCaT cells with TNF- α /IFN- γ will increase the levels of apoptosis, causing skin lesions.^{15–18}

Rhizoma coptidis (RC) is a widely used herb in traditional Chinese medicine. Pharmacological researches showed that RC has anti-inflammatory,¹⁹ anti-tumor,²⁰ anti-bacterial,²¹ hypoglycemic²² and other pharmacological effects. RC also could be used to treat eczema by external application.²³

At present study, we isolated alkaloids from RC and performed MTT assays to compare their inhibition against HaCaT. And then, the effects of target alkaloids on apoptosis of the keratinocytes stimulated with TNF- α /IFN- γ by flow cytometry, followed by verifying its inhibition on DNCB-induced AD mice and exploring its potential mechanisms.

2. Materials and methods

2.1. Reagents and animals

RC was obtained from Good Agricultural Practices Demonstration Base in Shizhu (Chongqing, China). Antibodies were from the Proteintech Group, Inc. (Wuhan, China) and Cell Signaling Technology, Inc (Boston, USA) respectively. 2,4-Dinitrochlorobenzene (DNCB), Wu Dai ointment^h (WDO) was from (Ziguang Guhan Group Hengyang Pharmaceutical Co., Ltd., China). TNF- α /IFN- γ were purchased from Leto Laboratories. Bid, cytochrome C, cathepsin B, caspase-3, caspase-7, caspase-8 and caspase-9 were purchased from Cell Signaling Technology, Inc (Boston, USA). IL-4 and IgE 7-week-old female BALB/c mice came from Weitonglihua Co., LTD (Beijing, China) and kept in a pathogen-free environment at Southwest University (Chongqing, China) according to institution regulations. The Southwest University Animal Care and Use Review Committee approved of all animal work (SCXK-YU 2015-0002), consistent with the 2011NIH guidelines for the care and use of laboratory animals.

2.2. Preparation of *Coptis chinensis* alkaloids

The air-dried rhizoma of *Coptidis chinensis* (10 kg) was pulverized and soaked with 95% ethanol (30 L $\times$ 5 d $\times$ 4) at room temperature. The extract was concentrated to give a residue (2.1 kg). The residue was suspended in H₂O (2 L) and then successively extracted with petroleum ether (2 L $\times$ 5), dichloromethane (2 L $\times$ 5), *n*-butanol (2 L $\times$ 5). The dichloromethane fraction (230.8 g) was separated over silica gel column eluted with CH₂Cl₂-MeOH (100:0–1:1) to yield 17 fractions (Frs. 1–17). A portion of the precipitates from Fr. 12 (12.3 g) was purified by Pre-HPLC (MeOH:H₂O, 20:80) to give compound 1 (340 mg), compound 2 (623 mg) and compound 3 (580 mg). Fr. 14 (34.8 g) was repeatedly chromatographed over silica gel column (CH₂Cl₂-MeOH, 10:1–1:1) to give compound 4 (1.5 g). The *n*-butanol fraction (980.1 g) was subjected to D101 macroporous resins column, followed by elution gradiently with deionized water, 30% MeOH, 60% ethanol to yield three fractions (A–C). Fr. B (378.2 g) was added with NaCl to a concentration of 20% to afford precipitates (110.4 g). A portion of the precipitates were separated over silica gel column eluted with (CH₂Cl₂-MeOH, 10:1–1:1), which led to the isolation of compound 5 (610 mg) and compound 6 (431 mg). Fr. C (149.5 g) was dissolved in water, then adjusted the pH value to 1 by HCl, followed by crystallization with MeOH to obtain compound 7 (884 mg).

2.3. Cell viability and apoptosis assay

Human skin keratinocyte (HaCaT) purchased from Shanghai Allwin-Biltechnology (Shanghai, China) and cultured in DMEM (Gibco, New Zealand) containing 10% fetal bovine serum (FBS; Gibco),

supplemented with penicillin and streptomycin (Invitrogen, USA). The cells were cultured in a humidified 5% CO₂ incubator at 37 °C.

HaCaT cells were plated 6000 cells/well in 96-well and cultured for 24 h before being treated with various concentrations of coptis alkaloids for 24 h. The cell viability was analyzed with MTT assays.²⁴

HaCaT cells were stimulated with or without TNF- α /IFN- γ (each 10 ng/ml) and treated with alkaloids for 24 h. Then, the cells were stained with FITC-conjugated Annexin-V and PI for flow cytometry analysis.²⁴

2.4. The treatment of RC alkaloids on DNCB-induced dermatitis mice

Mice were divided into five groups (n = 8): Normal controlⁱ (NC), atopic dermatitis control (AD), positive control (Wu Dai ointment, WDO), *Rhizoma coptidis* extract (RCE^j, 1 g/ml), MAG (0.1 g/ml). The RCE and MAG were dissolved in distilled water and were topically applied on the dorsal back skins of mice in a volume of 200 μ l/mouse. In NC group, vehicle (3:1 mixtures of acetone and olive oil) was topically applied instead of DNCB. Except NC, other four groups were treated with DNCB two times to obtain AD-like skin inflammation.^{25–29} For the first induction, the backs of the mice were shaved and challenged with 100 μ l of 2% DNCB in acetone/olive oil (3:1) for four days. Six days after first treatment, 0.5% DNCB was painted onto the backs of the mice every four days. Mice with AD symptom were randomly divided into four groups. The mice in RCE, MAG and WDO groups were treated with RCE, MAG and WDO once daily for 16 days respectively.

At the end, mice were sacrificed after measuring the weight and organs, and dorsal skin were collected and fixed with formalin for histological observation and detected Caspase-3 expression with previous methods.³⁰

The blood was collected for measuring IgE/IL-4 level using the commercial ELISA kit (Wanlei Biological Co., Ltd. Shenyang, China) with the manufacture's instruction.

The severity scoring of the skin was estimated one time per week. The atopic dermatitis severity index was evaluated by previous method.^{31,32} Mouse from each group was placed individually in a routine polycarbonate mouse cage and their behavior was monitored for 10 min after DNCB sensitization or administration.³³ A series of one or more biting movements was counted as one episode that ended when the mouse returned to the straight-forward position.

2.5. Hoechst staining

HaCaT cells were plated in a 12-well plate followed by treatment with three concentrations of MAG (500 μ g/ml, 1000 μ g/ml and 1500 μ g/ml) for 24 h and was analyzed with previous methods.²⁴

2.6. Detection of mitochondrial membrane potential

HaCaT cells were treated with MAG (500 μ g/ml, 1000 μ g/ml, 1500 μ g/ml) and stimulated with TNF- α /IFN- γ (10 ng/ml) at 37 °C for 24 h and was detected with previous methods.³⁴

2.7. Protein extraction and Western blot

The cells were treated with MAG (500 μ g/ml) and stimulated with TNF- α /IFN- γ (10 ng/ml) at 37 °C for 24 h. The expressions of caspase-3/7/8/9, Bid, Cyte C, CTSB in HaCaT cells and mice skin was analyzed by Western blotting with previous methods.²⁴

ⁱ NC: Normal control

^j RCE: *Rhizoma coptidis* extract

^h WDO: Wu Dai ointment

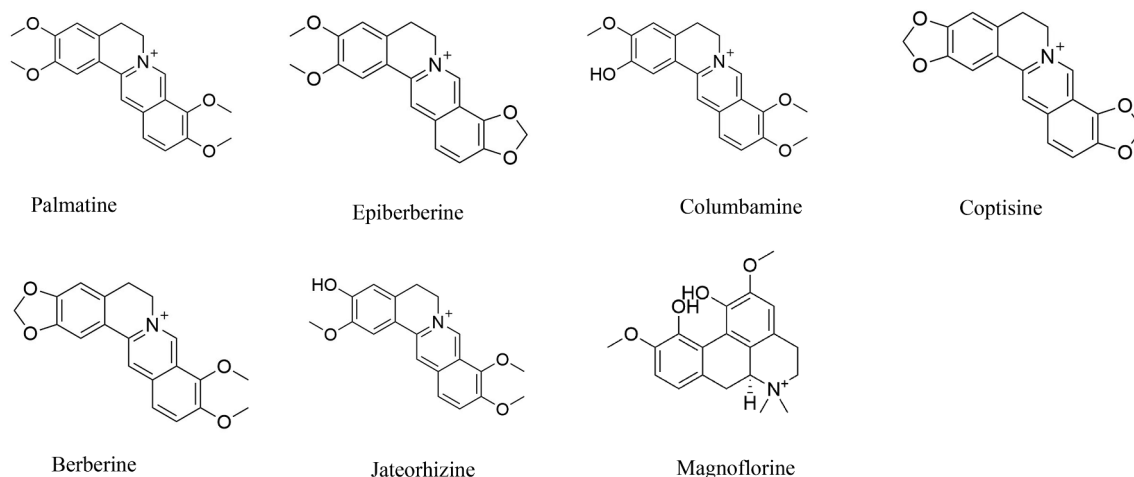


Fig. 1. The chemical structures of bioalkaloids in *Coptis chinensis* French.

2.8. Statistical analysis

All data are means $\pm$ standard deviation (SD). One-way analyses of variance (ANOVA) was performed to compare multiple variables, while Student's *t*-tests were used for comparison of variable pairs via SPSS software (version 20.0). *P* values with *P* < 0.05 was the threshold for a statistical difference while *P* < 0.01 was the threshold for a significant difference. The graphs were generated with Prism.

3. Results

3.1. Structure elucidation of isolated compounds

Compound 1 was jateorhizine (340 mg),³⁵ compound 2 was epiberberine (623 mg),³⁵ compound 3 was coptisine (580 mg),³⁵ compound 4 was magnoflorine (1.5 g),³⁶ compound 5 was palmatine (610 mg),³⁷ compound 6 was columbamine (431 mg)³⁸ and compound 7 was berberine (884 mg)³⁷. These known compounds (Fig. 1) were identified by comparison of their NMR and data (Figs. S8–S21) with reported in the literatures.

3.2. Magnoflorine showed the best activity on inhibiting IFN- γ and TNF- α -induced apoptosis of HaCaT cell

From Fig. 2A, the MTT assay showed that the alkaloids half maximal inhibitory concentrations were low and significant (*p* < 0.01) except MAG. Next, coptidis alkaloids were selected the optimum concentrations for subsequent examinations. As shown in Fig. 2B, MAG inhibited apoptosis in a dose dependent manner. After treated with MAG (1500 μ g/ml) the apoptotic rate of HaCaT cells was significantly decreased to 22.1% compared with 42.9% of model control. Meanwhile, other alkaloids promoted HaCaT cells apoptosis significantly in different degrees (Figs. S1–S6).

3.3. Magnoflorine attenuated DNCB-induced atopic dermatitis-like symptoms

The dorsal skin of the atopic dermatitis-model mice showed severe erythema, erosion and dryness that had been ameliorated by treatment with MAG (Fig. 3A). As shown in Fig. 3C, AD treatment resulted in significant increase in the number of scratches (54.8 ± 19.2 scratches/10 min, *P* < 0.01) compared with NC (2.2 ± 1.8). The spleen index had gradually increased 23% in AD group compared with NC and MAG had no obvious toxic effect on organs. In parallel, the dermatitis score was significantly higher in AD group (3.9 ± 3.4 , *P* < 0.01) compared with NC. Then, in AD group the serum level of IL-4 was

435.4 ± 24.3 pg/ml and the level of IgE was 440.1 ± 20.1 pg/ml, which were higher than that in NC group 196.1 ± 9.2 pg/ml and 207.1 ± 6.6 pg/ml, respectively. Notably, the H&E staining result of AD group revealed the epidermal thickening, rete ridge, stratum corneum thickening (Fig. 3B) and epidermal thickness in AD group was 6-fold higher than that of MAG group.³⁹

3.4. Magnoflorine decreased the expression of caspase-3 in immunohistochemical analysis

From the Fig. 3D, the number of caspase-3 positive cells were significantly lower in NC (155.1 ± 74.8) compared with AD group (1054.9 ± 74.9). WDO group (870.7 ± 36.9) and RCE group (814.0 ± 38.1) had similar effect. Distinctly, MAG group decreased the expression of caspase-3 (509.8 ± 60.0 , *p* < 0.01) in skin tissue.

3.5. Magnoflorine increased mitochondrial membrane potential

A decline of the $\Delta\psi_m$ may be an early event in the process of apoptosis. From the Fig. 4B, the low $\Delta\psi_m$ of model group (15.36 ± 0.76) was higher than control (7.45 ± 0.76). However, MAG (500 μ g/ml) decreased significantly the low $\Delta\psi_m$ (12.66 ± 0.73 , *p* < 0.05 VS model) and in a dose dependent manner.

3.6. Magnoflorine inhibits HaCaT cells apoptosis by attenuating the expressions of apoptosis-related proteins

As shown in Fig. 4A, the cells of the model group were shrinkage and the fluorescence intensities of nuclei was increased. However, the nuclei were round, with sharp edges and uniform staining were observed upon MAG treatment. As the concentrations of MAG increased, the number of apoptotic cells were decreased. Furthermore, the results indicated that there was a marked increase in the level of Bid, Cyte C, CTSE, and caspase-3/7/8/9 expressions in model group compared with control group (Fig. 4C). However, the group treated with TNF- α /IFN- γ (each 10 ng/ml) and added the MAG (500 μ g/ml) decreased all expressions of them distinctly. Furthermore, MAG reduced the expression of caspase-8 in skin (Fig. S7A).

4. Discussion

AD is characterized by eczematous lesions.⁴⁰ Most AD patients are increased serum immunoglobulin E (IgE) and accompanied by the release of some inflammatory factors.^{41–43} The main mechanisms of AD are abnormalities of epidermal structure and function, another is inappropriate immune responses to antigens encountered in the skin,^{2,44}

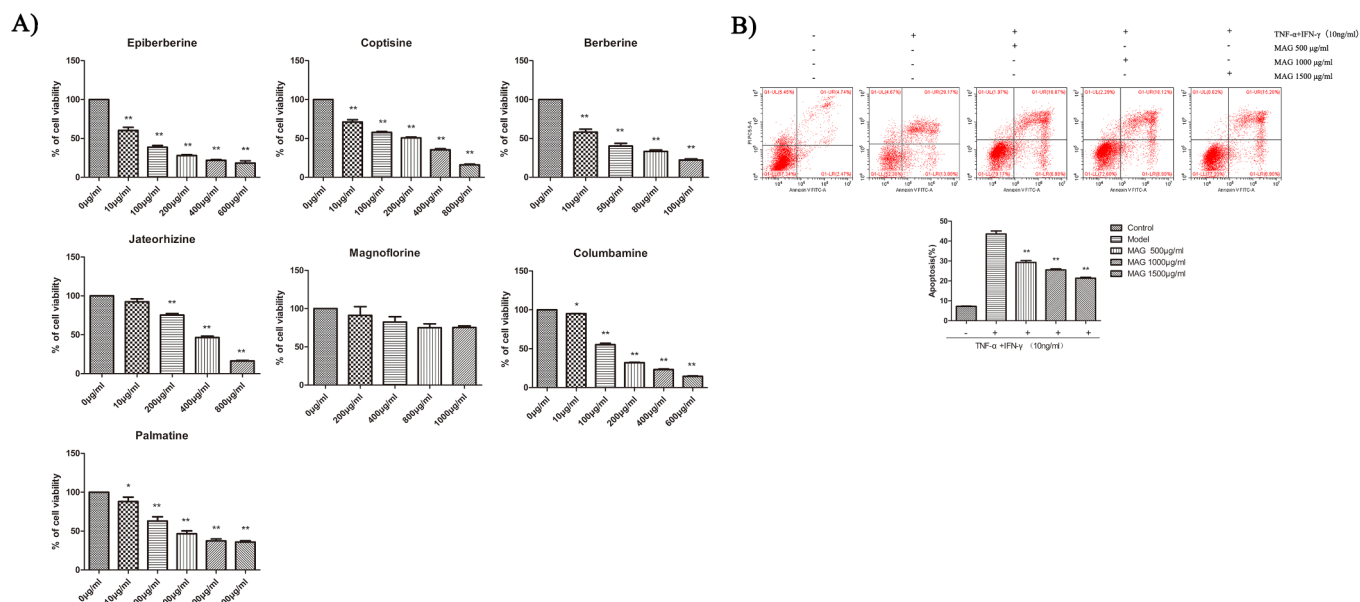


Fig. 2. MAG showed the best activity on inhibiting IFN- γ and TNF- α -induced apoptosis of HaCaT cell. A) MTT results of seven alkaloids. B) Flow cytometry analysis of MAG. Each point represents the mean $\pm$ SD of three independent experiments (* $P < 0.05$, ** $p < 0.01$ vs model group). MAG: magnoflorine.

but it still remains debate of this topics. Despite all this, it is clearly that skin barrier function and immunity are closely related.⁴⁴ Moreover, previous research has demonstrated that mRNA expression analyses revealed differential expression of apoptosis-related genes in atopic dermatitis keratinocytes and skin and the upregulation of immune system-related genes.¹⁶ It has reported that T cell-mediated

keratinocyte apoptosis plays a key role in the formation of eczema dermatitis and increased apoptosis in keratinocytes is a major cause of common atopic eczema, upregulation of Fas from keratinocytes by IFN- γ released from activated T cells makes HaCaT cells susceptible to apoptosis.^{16,45,15} Meanwhile, it has proposed that inflammatory infiltrate is not the consequence but the cause of apoptosis.⁵ In addition, it

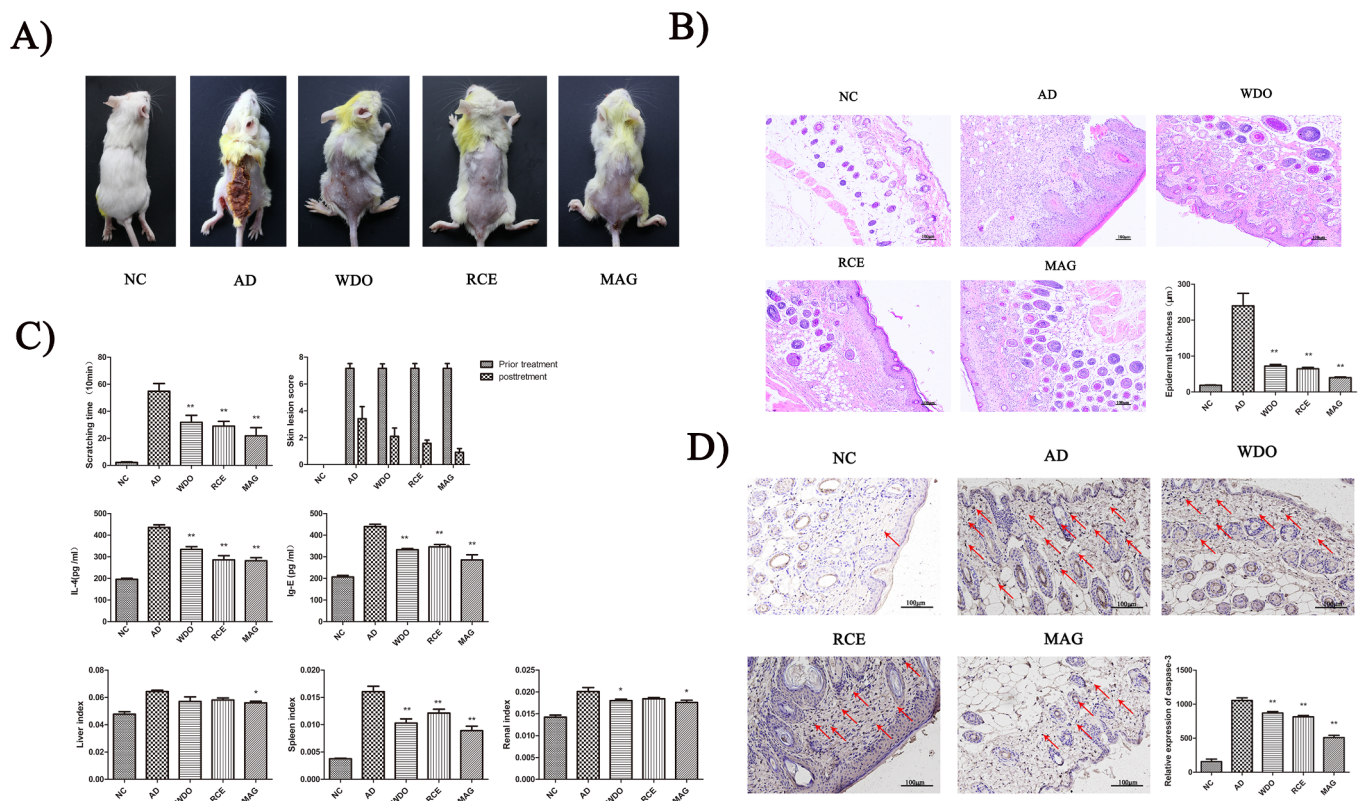


Fig. 3. MAG attenuated DNCB-induced atopic dermatitis-like symptoms. A) Mice treated with DNCB induced AD symptoms and divided into different group. B) Hematoxylin and eosin staining results of skin tissues. C) Behavioral and biochemical indicators. D) Comparison of immunohistochemical analysis between groups. The red arrows shows high immunostaining for caspase-3, which is brown. Data are expressed as the mean $\pm$ SD. (* $P < 0.05$, ** $p < 0.01$ VS AD). NC: Normal control AD: Atopic dermatitis RCE: *Rhizoma coptidis* extract. WDO: Wu Dai ointment. MAG: magnoflorine.

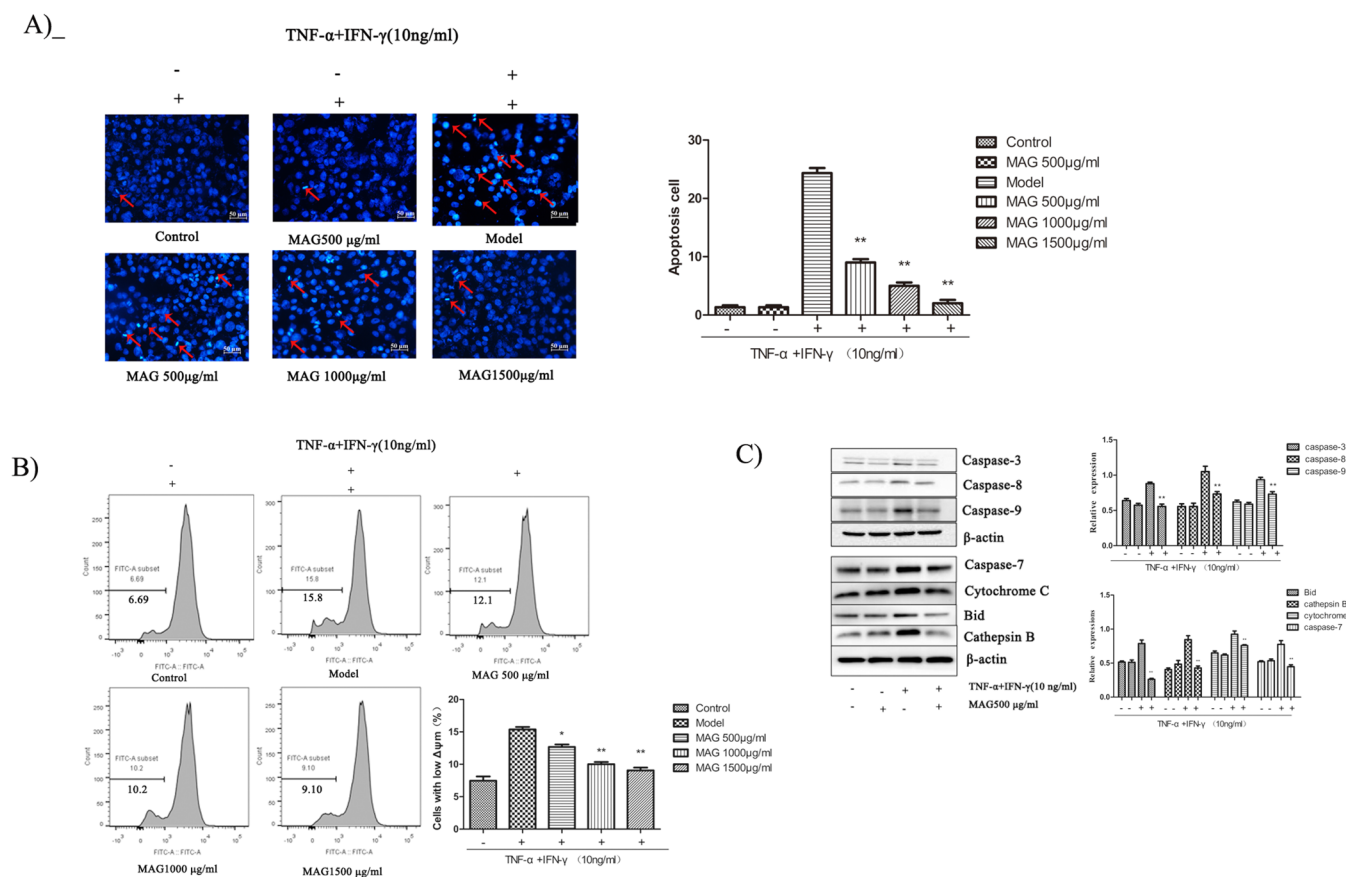


Fig. 4. MAG inhibited apoptosis by blocking $\Delta\psi\text{m}$ loss and attenuated the expressions of apoptosis-related proteins. A) Hoechst staining. MAG protects the HaCaT cells from $\text{INF-}\gamma/\text{TNF-}\alpha$ induced apoptosis. B) MAG induced increase of $\Delta\psi\text{m}$. C) Western blot. Effect of MAG inhibited the expressions of caspase-3/7/8/9, Bid, CTSB and Cyte C. The data are presented as mean $\pm$ SD of the three experiments. (* $p < 0.05$, ** $p < 0.01$ VS model group). MAG: magnoflorine. $\Delta\psi\text{m}$: mitochondrial membrane potential. CTSB: cathepsin B. Cyte C: cytochrome C.

was shown that $\text{TNF-}\alpha/\text{INF-}\gamma$ activated human keratinocytes was used for inflammatory model,^{46–48} therefore, it can induce apoptosis in some extent. Hence, finding a substance that can inhibit the apoptosis of keratinocytes may play a vital role in relieving atopic dermatitis.

In this study, separation and extraction of coptidis alkaloids on the basis of pre-laboratory.⁴⁹ Seven alkaloids were extracted and separated, including coptisine, berberine, epiberberine, jateorhizine, columbamine, palmatine and magnoflorine. From the MTT results we found that the alkaloids afforded significantly cells toxicity except MAG (Fig. 2). Analyzed by flow cytometry, MAG was the only one that inhibited apoptosis of HaCaT cells during the seven alkaloids. Next, the effect of RCE and MAG in the treatment of AD was verified *in vivo*. Topical application of DNCB in mice is commonly used to induce AD-like skin lesion.^{25,50–52} It is well known that AD is leading to eczematous and itchy lesions.⁵³ Skin severity and scratching behaviors were significantly reduced from our data (Fig. 3). In our comparative experiments, the efficacy of the MAG in treating AD-like lesion was better than WDO and RCE, and similar to WDO in scratching behaviors. It is suggested that the topical treatment of MAG could alleviate the clinical symptoms of AD. On the last day, the morphology and weight of spleen dissected from mice were observed and measured. From the result, the AD group exhibited 4.2-fold higher spleen index rather than that of NC ($p < 0.01$). The spleen index all three group (WDO, REC and MAG) were significantly lower than that of AD group ($p < 0.01$). It may also be a change in the composition of T cells and dendritic cells.⁵⁴ The comparison between the REC group, MAG group and AD group shown that other alkaloids in coptis may also played a certain anti-inflammatory effect, like berberine, but MAG had played an important role. Notably, AD group developed severe hyperplasia, inflammatory

cell infiltration and disappear of hair follicles, but NC reduced epidermal keratinization and dermal edema, hair follicle sebaceous glands were not affected by inflammatory infiltration (Fig. 3). MAG and WDO groups were similar with NC from the result. It implied that the topical application of MAG could alleviated the AD symptoms in the clinical aspects. The reduction of skin thickness in groups could be supported by the data in mice. Beyond that, the expression of caspase-8 was inhibited in the skin of mice (Fig. S7A). From the comparison of immunohistochemical analysis between groups (Fig. 3D), MAG had decreased the expression of caspase-3 in skin tissue. Therefore, it is presumed that MAG may inhibited apoptosis

Additionally, in order to verify the MAG has the ability to inhibit apoptosis on HaCaT cells, Hoechst staining was performed. It exposed that the use of MAG had no obviously apoptosis effect on cells without treated with $\text{TNF-}\alpha/\text{INF-}\gamma$ when compared with control (Fig. 4A). However, compared with the model group, the number of apoptotic cells treated with MAG was significantly reduced on the basis of the model. Moreover, in order to find the mechanism of MAG inhibiting apoptosis, $\Delta\psi\text{m}$ detection and western blotting experiments were performed. From the result, MAG reduced the low $\Delta\psi\text{m}$ and blocked the $\Delta\psi\text{m}$ loss (Fig. 4B). MAG suppressed the express of Bid, CTSB, Cyte C and caspase-3/7/8/9 in $\text{TNF-}\alpha/\text{INF-}\gamma$ stimulated HaCaT cells (Fig. 4C, $p < 0.01$). Meanwhile, in the signal transduction of apoptosis, the caspase family plays an important role, is the convergence point of multiple signaling pathways, and the ultimate pathway for performing apoptosis^{55,56} and Bid is a key molecule linking the exogenous death receptor and the endogenous mitochondrial pathway, so whether the cell is mediated by exogenous or endogenous pathways is related to the regulation of Bid expression.⁵⁷ Moreover, Bid is an important molecule

of lysosome-mediated apoptosis and can trigger the release of Cyte C from mitochondria. Apoptosis is also accompanied by a decrease in $\Delta\psi_m$ to some extent. From our data, model group exposed high expressions of Bid, CTSB, Cyte C, caspase-3/7/8/9 which mean stimulating HaCaT cells with TNF- α /IFN- γ rendered them susceptible to apoptosis. However, when giving MAG based on the basis of the model group, the expressions of them were significantly decreased ($p < 0.01$, VS model group) and no significant difference from the control. Thus further proved that apoptosis of HaCaT cells induced by TNF- α /IFN- γ may relate to CTSB and MAG inhibited apoptosis by decreasing the expressions of CTSB, Bid, Cyte C and caspase-3/7/8/9.

In conclusion, in this study, the effect of *Rhizoma coptidis* on AD was verified by *in vitro* and *in vivo* experiments, and the material basis for inhibiting apoptosis of keratinocytes was found by cell experiments. MAG achieves the effect of inhibiting apoptosis by reducing the expressions of key proteins controlling apoptosis in the CTSB, Bid, Cyte C and caspase family. Overall, these results further increase the potential of *Rhizoma coptidis* in treating atopic dermatitis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Siqi Wu wrote this article. Xuegang Li and Xiaoli Ye provided the article ideas. All the authors participated in the experiment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmc.2019.115093>.

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Supplement

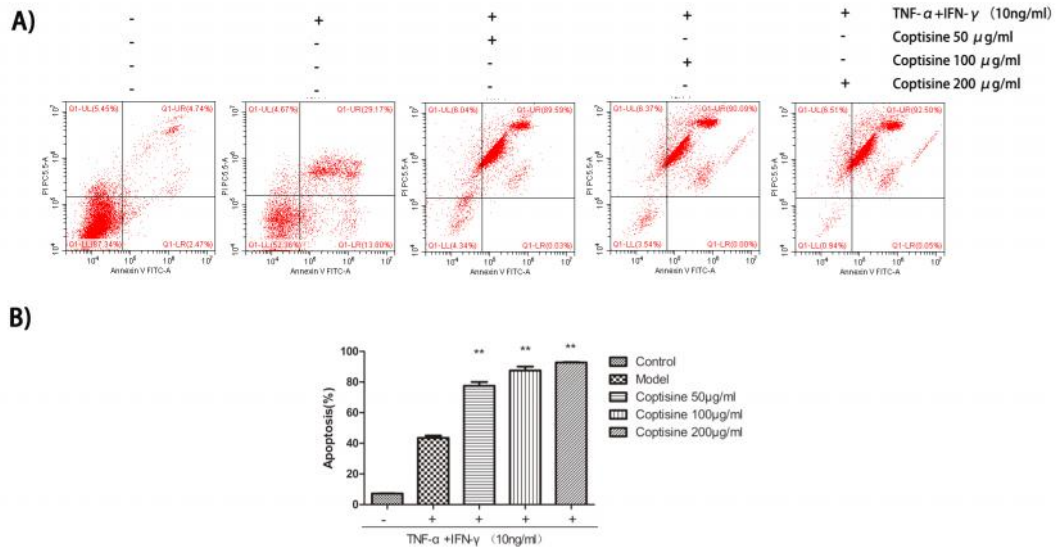


Fig.S1 Coptisine promote apoptosis in HaCaT cells induced by IFN- γ and TNF- α . Cells were stimulated with or without IFN- γ and TNF- α (each 10ng/ml) and treated with coptisine for 24h. (A) The cells were stained with FITC-conJugated Annexin-V and PI for flow cytometry analysis. (B) The percentages of apoptotic cells were determined by counting the percentage of Annexin-v-positive cells. Each point represents the mean $\pm$ SD of three independent experiments (**p<0.01 vs model group)

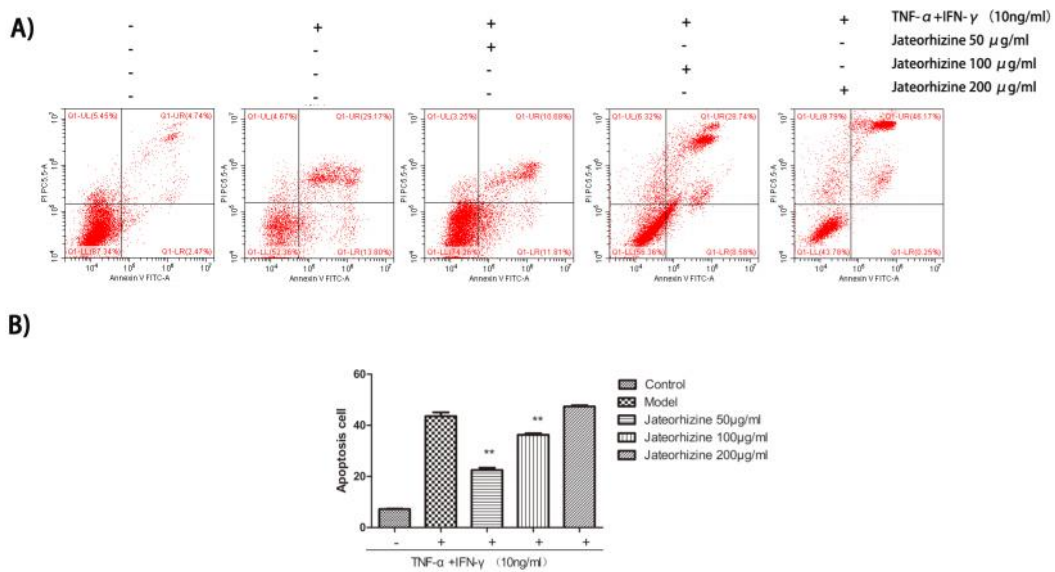


Fig.S2 Jateorhizine promote apoptosis in HaCaT cells induced by IFN- γ and TNF- α . Cells were stimulated with or without IFN- γ and TNF- α (each 10ng/ml) and treated with Jateorhizine for 24h. (A) The cells were stained with FITC-conJugated Annexin-V and PI for flow cytometry analysis. (B) The percentages of apoptotic cells were determined by counting the percentage of Annexin-v-positive cells. Each point represents the mean $\pm$ SD of three independent experiments (**p<0.01 vs model group)

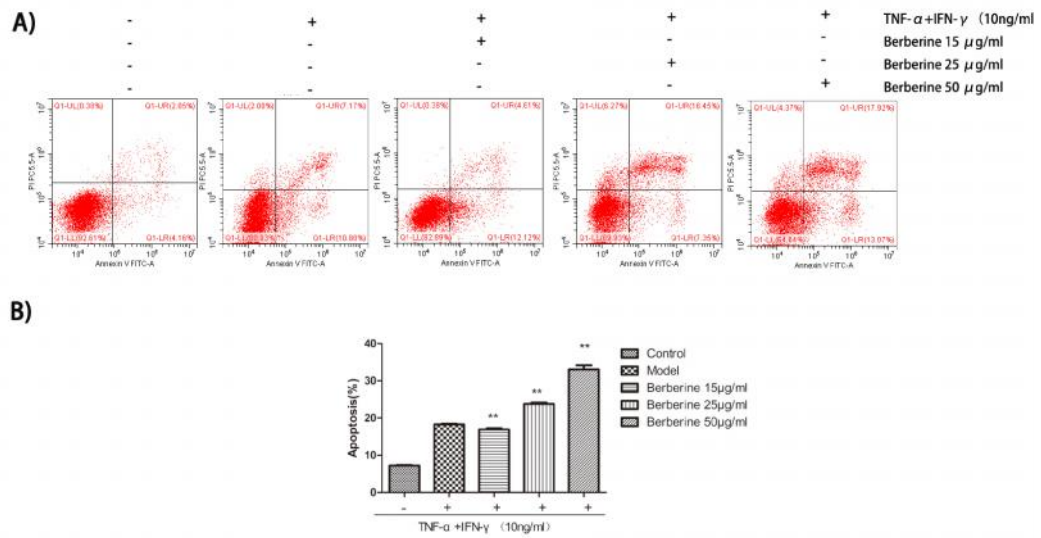


Fig.S3 Berberine promote apoptosis in HaCaT cells induced by IFN- γ and TNF- α . Cells were stimulated with or without IFN- γ and TNF- α (each 10ng/ml) and treated with berberine for 24h. (A) The cells were stained with FITC-conJugated Annexin-V and PI for flow cytometry analysis. (B) The percentages of apoptotic cells were determined by counting the percentage of Annexin-v-positive cells. Each point represents the mean $\pm$ SD of three independent experiments (**p<0.01 vs model group)

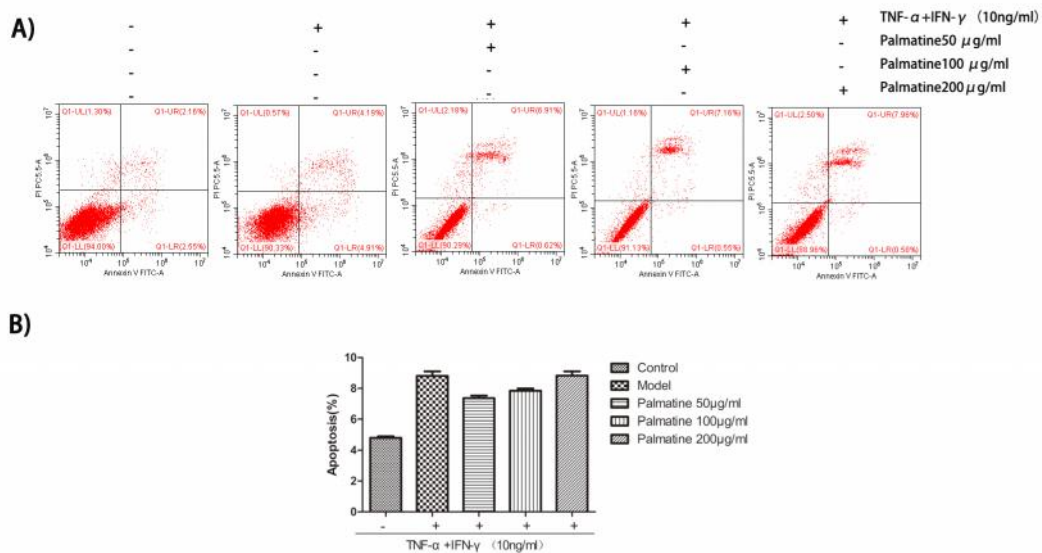


Fig.S4 Palmatine promote apoptosis in HaCaT cells induced by IFN- γ and TNF- α . Cells were stimulated with or without IFN- γ and TNF- α (each 10ng/ml) and treated with palmatine for 24h. (A) The cells were stained with FITC-conJugated Annexin-V and PI for flow cytometry analysis. (B) The percentages of apoptotic cells were determined by counting the percentage of Annexin-v-positive cells. Each point represents the mean $\pm$ SD of three independent experiments (**p<0.01 vs model group)

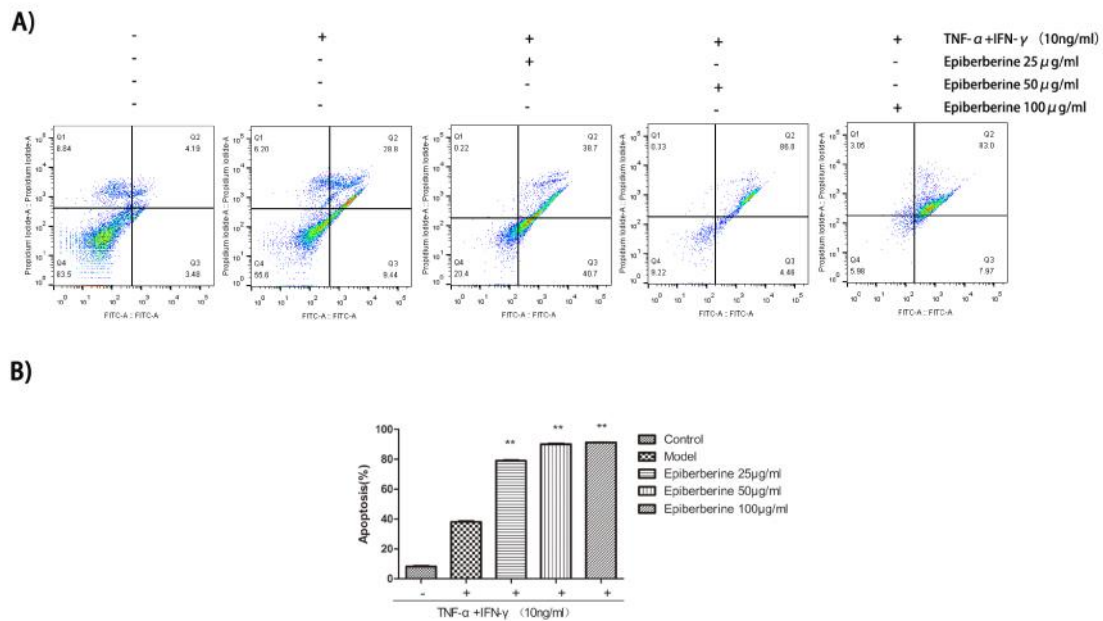


Fig.S5 Epiberberine promote apoptosis in HaCaT cells induced by IFN- γ and TNF- α . Cells were stimulated with or without IFN- γ and TNF- α (each 10ng/ml) and treated with epiberberine for 24h. (A) The cells were stained with FITC-conJugated Annexin-V and PI for flow cytometry analysis. (B) The percentages of apoptotic cells were determined by counting the percentage of Annexin-v-positive cells. Each point represents the mean $\pm$ SD of three independent experiments (**p<0.01 vs model group)

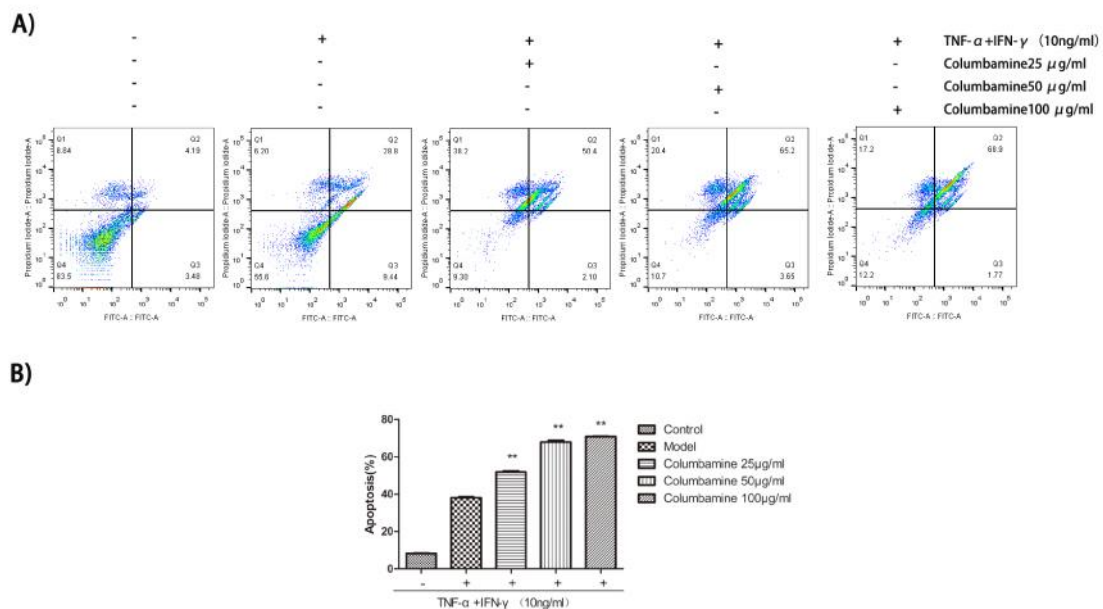


Fig.S6 Columbamine promote apoptosis in HaCaT cells induced by IFN- γ and TNF- α . Cells were stimulated with or without IFN- γ and TNF- α (each 10ng/ml) and treated with columbamine

for 24h. (A) The cells were stained with FITC-conJugated Annexin-V and PI for flow cytometry analysis. (B) The percentages of apoptotic cells were determined by counting the percentage of Annexin-v-positive cells. Each point represents the mean $\pm$ SD of three independent experiments (** $p < 0.01$ vs model group)

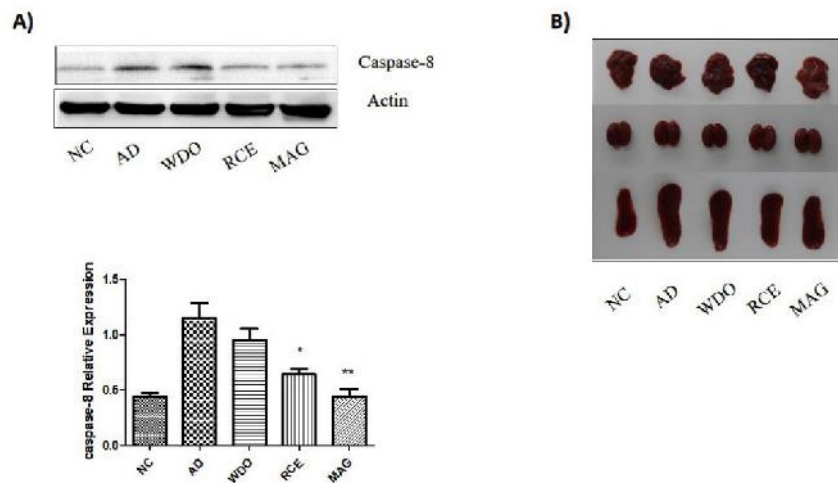


Fig.S7 Effect of MAG on mice. (A) The skin were obtained on the last day, the expression of caspase-8 were detected (B) Organs were collected on the last day and weighed.

Compound **1**: Jateorhizine ¹HNMR(400 MHz, CD₃OD) : δ 9.73(1H,s, H-8), 8.77(1H,s, H-13)8.10(1H,d, J =8 Hz,H-12),8.01(1H,d, J =8 Hz,H-11), 7.65(1H,s, H-1), 6.85(1H,s,H-4),4.61(2H,m,H-6),4.20(3H,s,9-OCH₃),4.10(3H,s,10-OCH₃), 4.02(3H,s,2-OCH₃), 3.22(2H,m,H -5); ¹³CNMR(100 MHz, CD₃OD): δ 151.8(C-9), 151.7(C-2), 149.6(C-3), 146.5(C-8), 145.8(C-10), 140.2(C-13a), 135.4(C-12a), 130.2(C-4a), 128.0(C-12), 124.3(C-11), 123.1(C-13b), 120.9(C-13), 119.3(C-8a), 115.8(C-4), 110.0(C-1), 62.5(9-OCH₃), 57.6(10-OCH₃), 57.3(2-OCH₃), 56.9(C-6), 27.6(C-5).

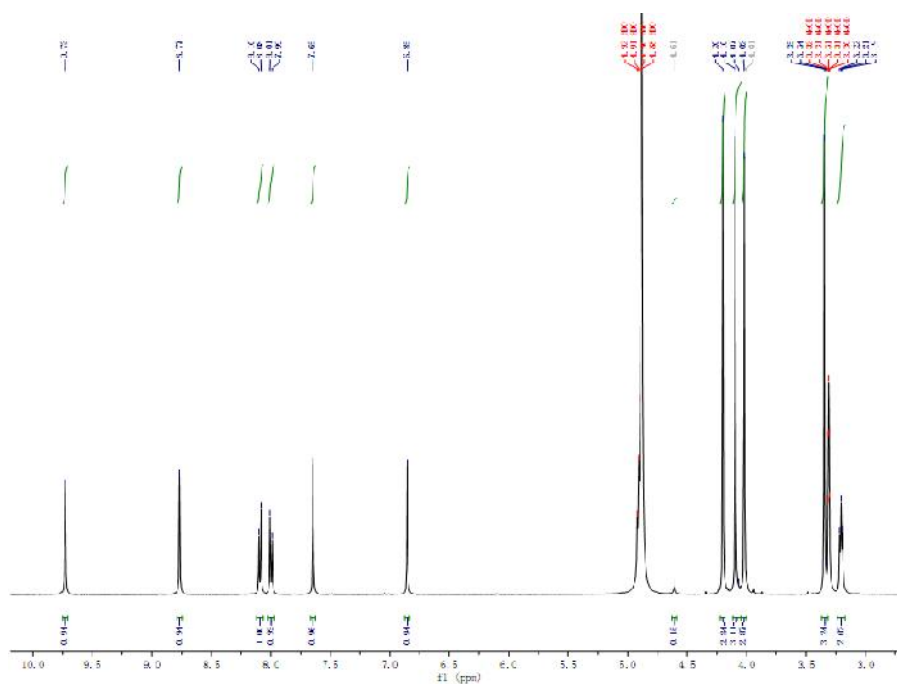


Fig.S8 ¹HNMR spectra of Jateorhizine

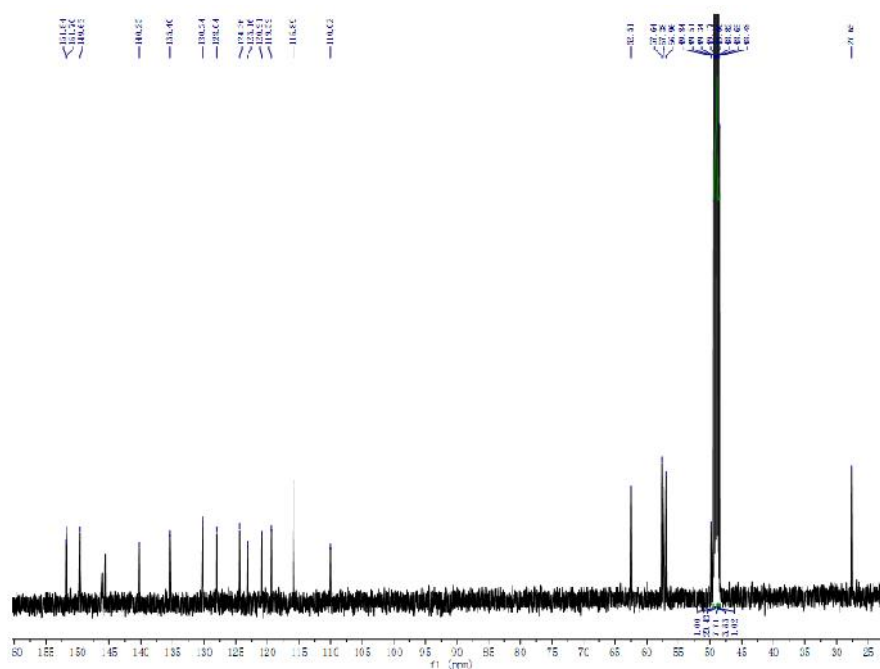


Fig.S9 ¹³CNMR spectra of Jateorhizine

Compound 2: Epiberberine ¹H NMR (400 MHz, CD₃OD): δ 9.32 (1H, s, H-8), 8.01 (1H, s, H-13), 7.63 (1H, d, *J*=8.0 Hz, H-12), 7.62 (1H, d, *J*=8.0 Hz, H-11), 7.40 (1H, s, H-1), 6.87 (1H, s, H-4), 6.31 (2H, s, -OCH₂O-), 4.70 (2H, m, H-6), 4.60 (3H, s, 2-OCH₃), 3.73 (3H, s, 3-OCH₃), 3.01 (2H, m, H-5); ¹³C NMR (100 MHz, CD₃OD): δ 152.1 (C-3), 152.0 (C-2), 149.8 (C-10), 146.3 (C-9), 145.7 (C-8), 135.1 (C-13a), 131.8 (C-12a), 128.0 (C-4a), 124.5 (C-12), 123.2 (C-11), 121.8 (C-13), 121.4 (C-13b), 112.2 (C-8a), 109.3 (C-4), 106.5 (C-1), 103.6 (-OCH₂O-), 62.5 (C-6), 57.6 (2-OCH₃), 57.3 (3-OCH₃), 27.7 (C-5).

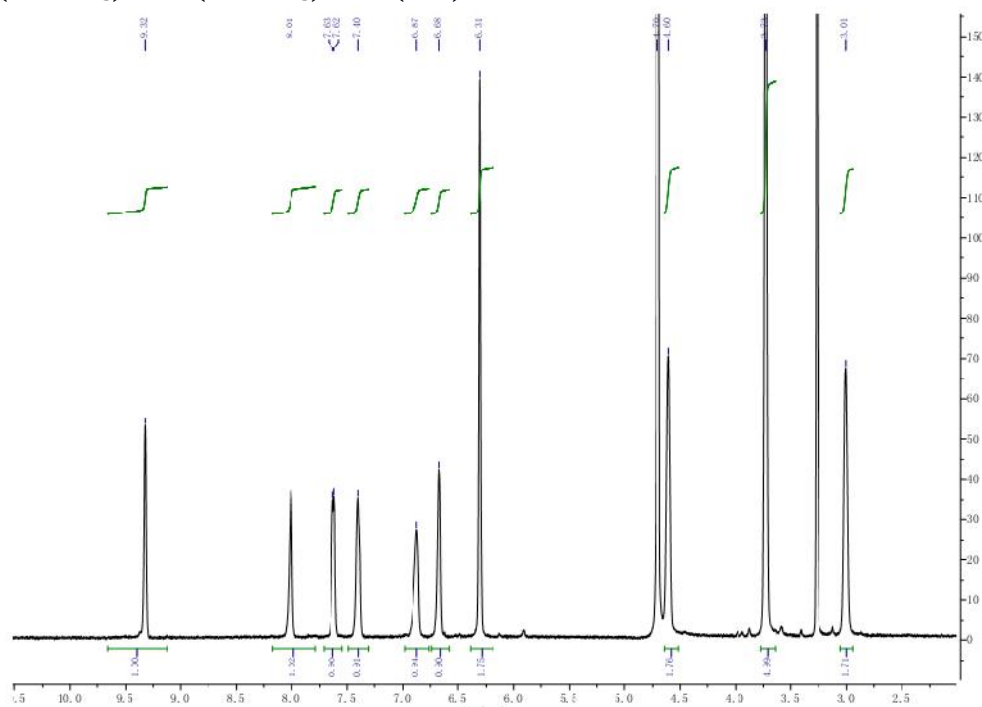


Fig.S10 ¹H NMR spectra of Epiberberine

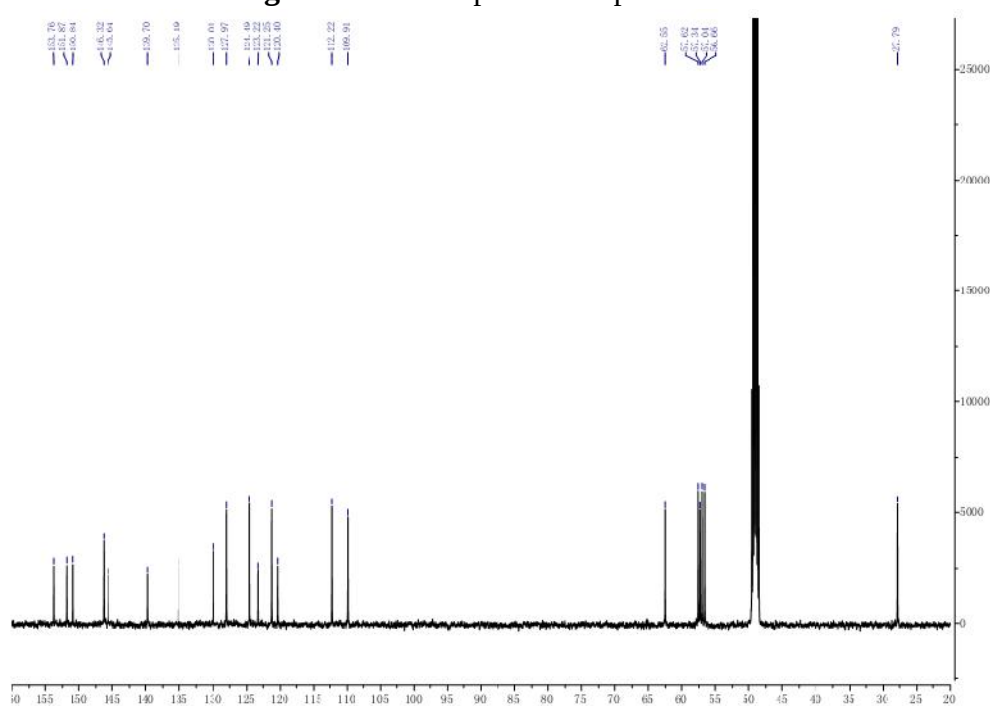


Fig.S11 ¹³C NMR spectra of Epiberberine

Compound **3**: Coptisine ^1H NMR(400 MHz, DMSO): δ 9.91(1H, s, H-8), 8.92(1H, s, H-13), 8.03(1H, d, $J=6.8$ Hz, H-12), 7.84(1H, d, $J=6.8$ Hz, H-11), 7.77(1H, s, H-1), 7.01(1H, s, H-4), 6.53(2H, s, $-\text{OCH}_2\text{O}-$), 6.17(2H, s, $-\text{OCH}_2\text{O}-$), 4.88(2H, m, H-6), 3.21(2H, m, H-5); ^{13}C NMR(100 MHz, DMSO): δ 149.8(C-10), 147.7(C-3), 147.1(C-2), 144.6(C-8), 143.9(C-9), 136.9(C-13a), 132.4(C-12a), 130.64(C-4a), 121.8(C-12), 121.0(C-13b), 121.0(C-13), 120.5(C-11), 111.7(C-8a), 108.5(C-4), 105.3(C-1), 104.5($-\text{OCH}_2\text{O}-$), 102.1($-\text{OCH}_2\text{O}-$), 55.2(C-6), 26.3(C-5).

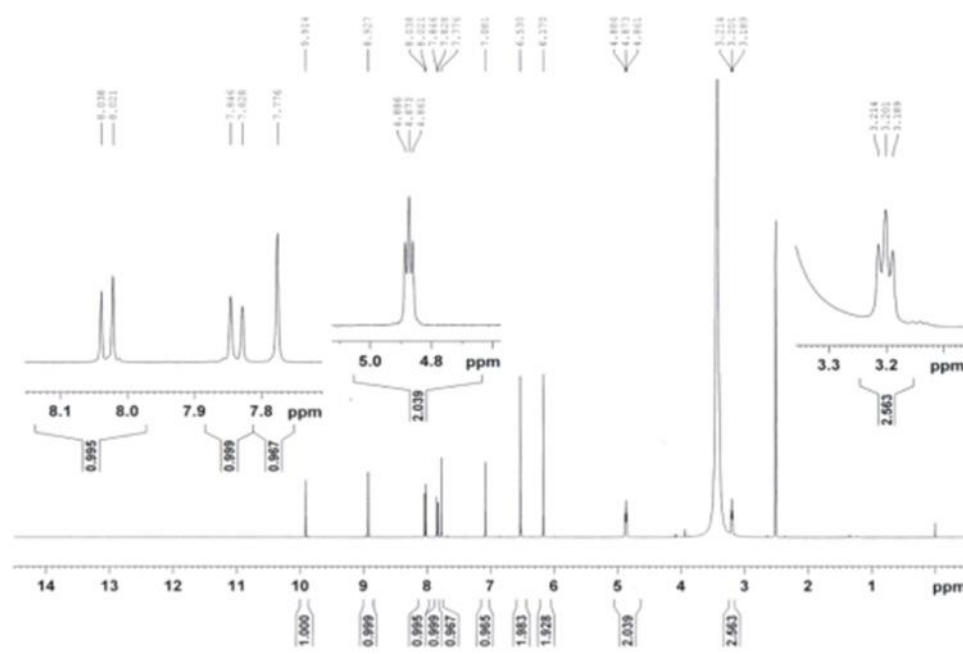


Fig.S12 ^1H NMR spectra of Coptisine

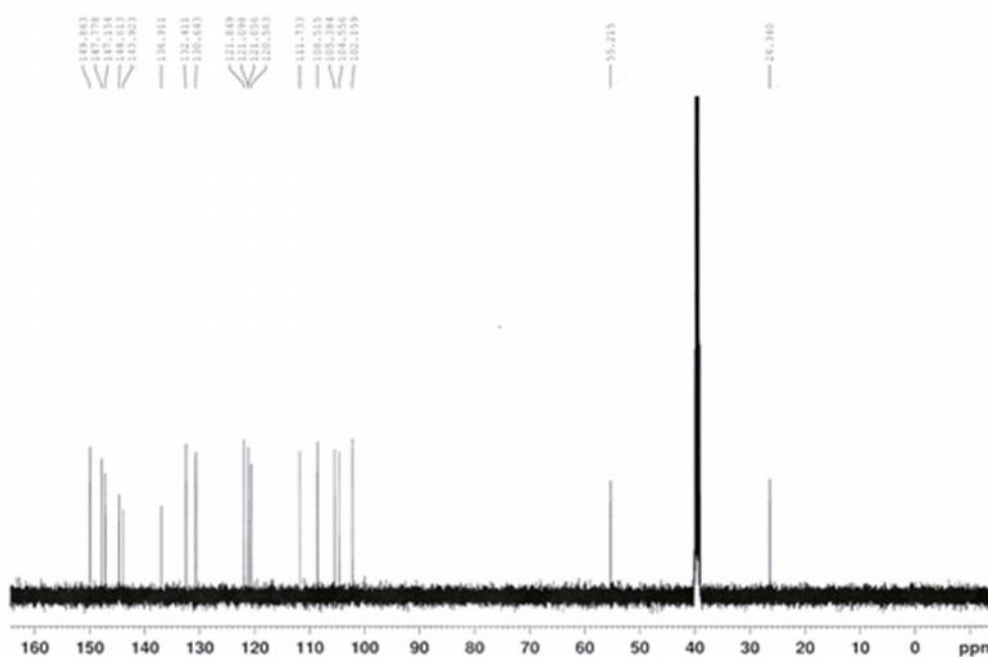


Fig.S13 ^{13}C NMR spectra of Coptisine

Compound **4**: Magnoflorine $^1\text{H NMR}$ (400 MHz, CD_3OD): δ 6.66(1H, d, $J=7.6$ Hz, H-9), 6.55(1H, d, $J=7.6$ Hz, H-8), 6.44(1H, s, H-3), 3.83(3H, s, 10- OCH_3), 3.75(3H, s, 2- OCH_3), 3.59(1H, m, H-6a), 3.42(1H, m, H-5 α), 3.23(3H, s, N- CH_3 - α), 3.14(1H, m, H-4 β), 3.11(1H, m, H-5 β), 2.90(1H, m, H-7 α), 2.77(3H, s, N- CH_3 - β), 2.58(1H, m, H-4 α), 2.43(1H, t, $J=12.0$ Hz, H-7 β). $^{13}\text{C NMR}$ (100 MHz, CD_3OD): δ 153.1(C-2), 151.7(C-10), 150.9(C-11), 149.9(C-1), 126.0(C-7a), 123.5(C-1a), 121.0(C-11a), 116.9(C-8), 115.7(C-3a), 110.7(C-9), 109.5(C-3), 71.1(C-6a), 62.3(C-5), 56.3(10- OCH_3), 56.0(2- OCH_3), 53.9(β -N- CH_3), 43.5(α -N- CH_3), 31.6(C-7), 24.6(C-4).

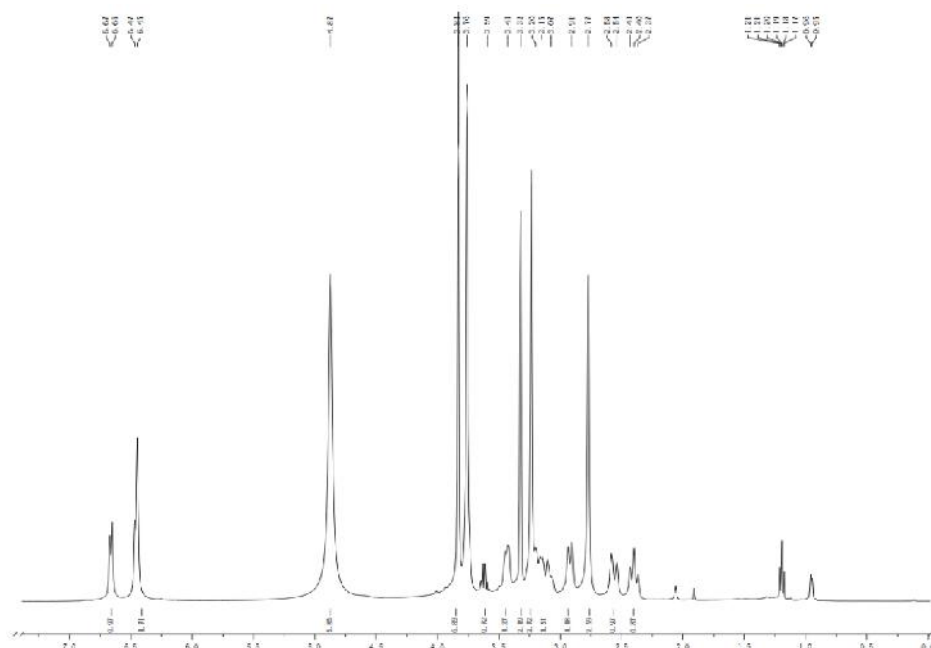


Fig.S14 $^1\text{H NMR}$ spectra of Magnoflorine

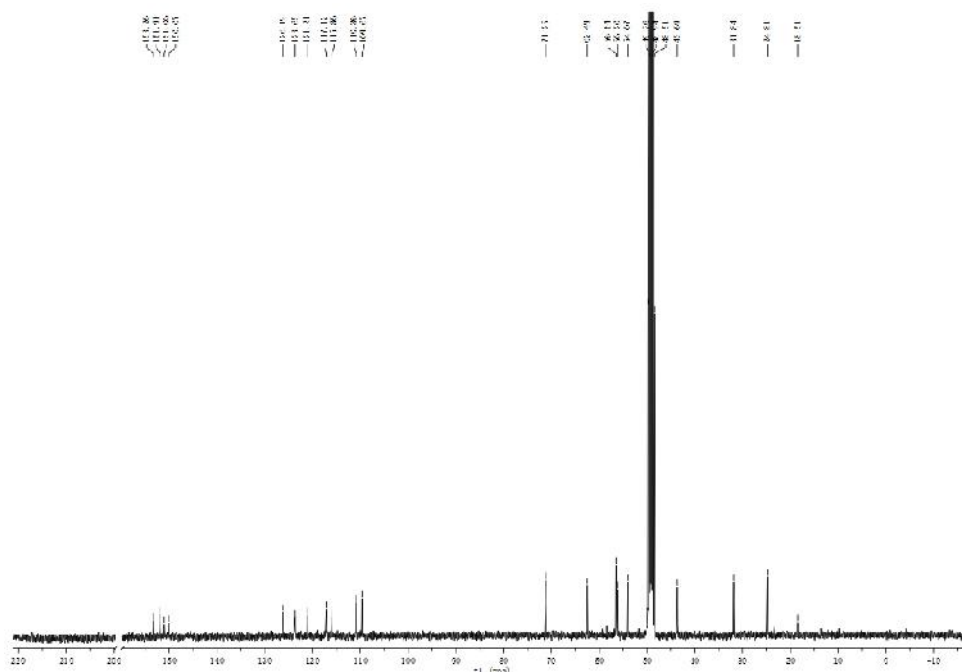


Fig.S15 $^{13}\text{C NMR}$ spectra of Magnoflorine

Compound 5: Palmatine ¹HNMR (400 MHz, CD₃OD): δ 9.74(1H, s, H-8), 8.78(1H, s, H-13), 8.09(1H, d, J=8.0 Hz, H-12), 8.01(1H, d, J=8.0 Hz, H-11), 7.62(1H, s, H-1), 7.03(1H, s, H-4), 4.95(2H, t, J=6 Hz, H-6), 4.20(3H, s, 9-OCH₃), 4.09(3H, s, 10-OCH₃), 3.98(3H, s, 2-OCH₃), 3.93(3H, s, 3-OCH₃), 3.30(2H, t, J=6 Hz, H-5); ¹³CNMR (100 MHz, CD₃OD): δ 153.7(C-9), 151.8(C-3), 150.8(C-2), 146.3(C-8), 145.6(C-10), 139.6(C-13a), 135.1(C-12a), 130.0(C-4a), 127.9(C-12), 124.4(C-11), 123.2(C-13b), 121.2(C-13), 120.3(C-8a), 112.2(C-4), 109.9(C-1), 62.5(9-OCH₃), 57.6(10-OCH₃), 57.3(2-OCH₃), 57.0(3-OCH₃), 56.6(C-6), 27.7(C-5).

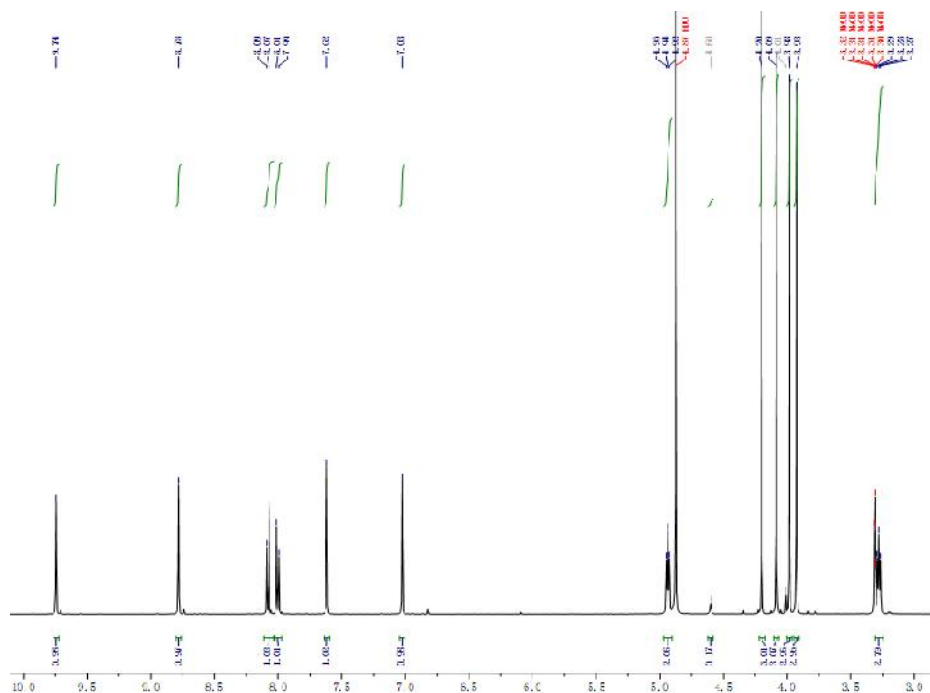


Fig.S16 ¹H NMR spectra of Palmatine

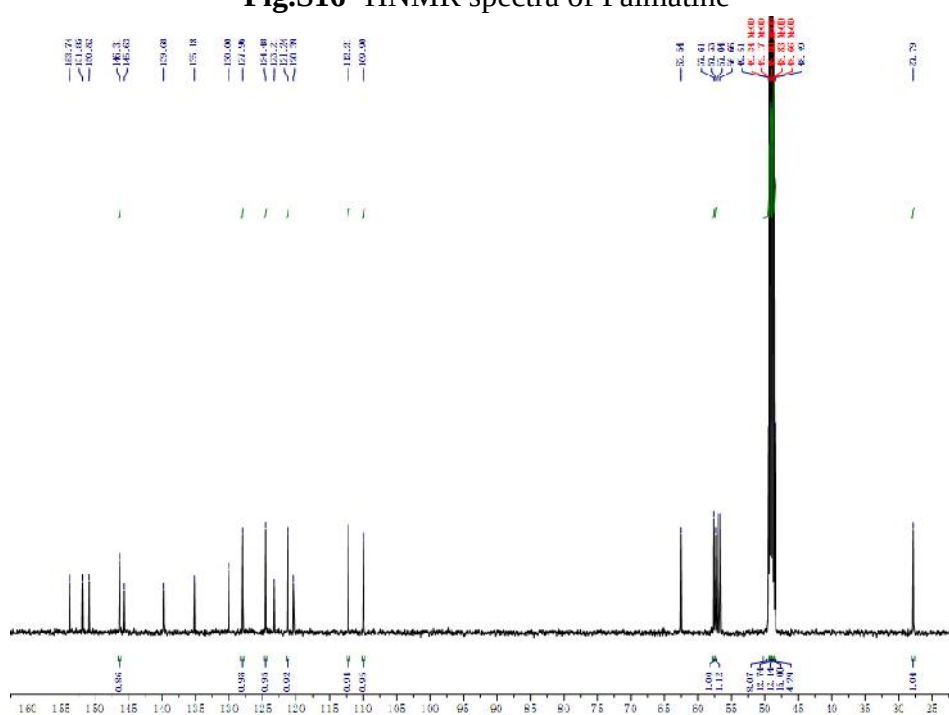


Fig.S17 ¹³C NMR spectra of Palmatine

Compound **6**: Columbamine ¹HNMR (400 MHz, CD₃OD): δ9.74(1H, s, H-8), 8.62(1H, s, H-13), 8.10(1H, d, *J*=0.1 Hz, H-12), 7.99(1H, d, *J*=9.1 Hz, H-11), 7.52(1H, s, H-1), 7.01(1H, s, H-4), 4.94(2H, t, *J*=6.2 Hz, H-6), 4.09(6H, s, 9-OCH₃), 3.96(3H, s, 3-OCH₃), 3.25(2H, t, *J*=6.2 Hz, H-5) ¹³CNMR(100 MHz, CD₃OD): δ152.5(C-2), 152.0(C-3), 148.3(C-10), 146.4(C-8), 145.8(C-9), 140.12(C-4a), 135.4(C-8a), 128.6(C-12), 128.2(C-11), 124.5(C-12a), 123.4(C-13a), 121.2(C-13), 120.8(C-1a), 113.3(C-4), 112.1(C-1), 62.6(9-OCH₃), 57.8(10-OCH₃), 57.6(3-OCH₃), 56.8(C-6), 27.9(C-5).

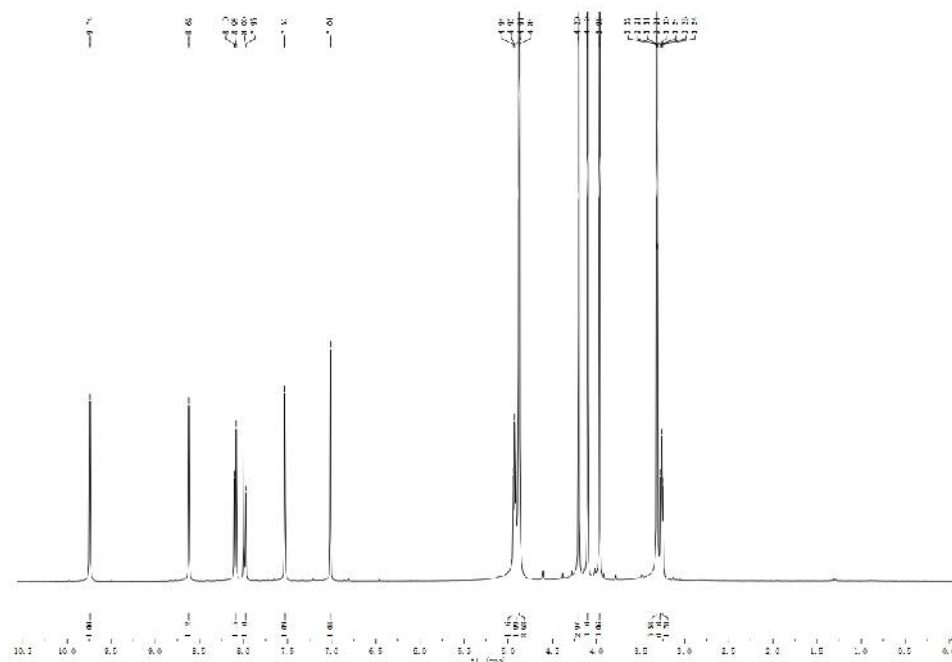


Fig.S18 ¹HNMR spectra of Columbamine

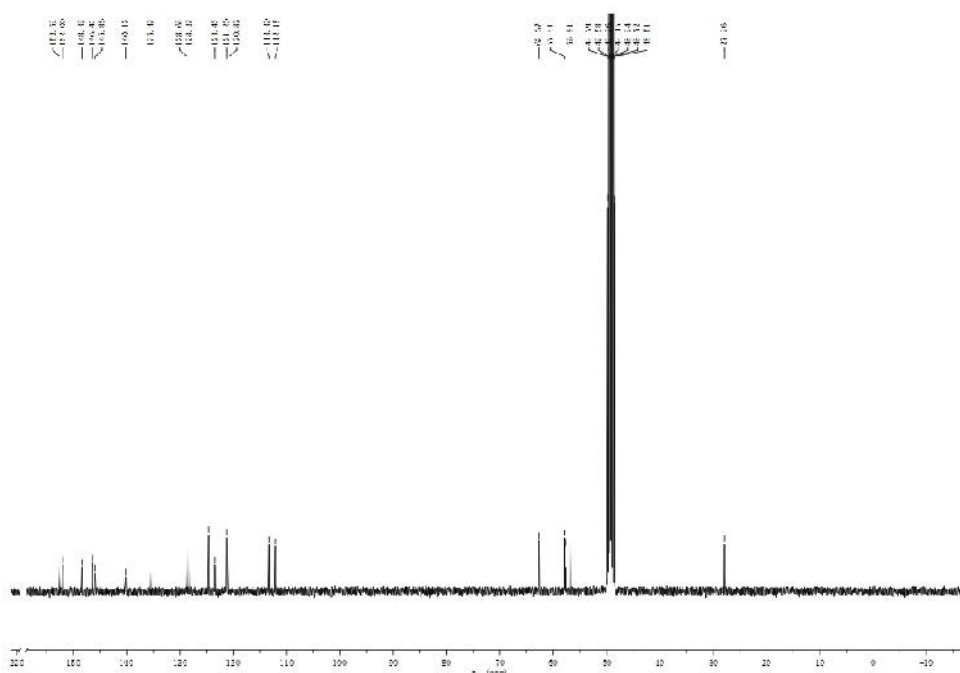


Fig.S19 ¹³CNMR spectra of Columbamine

Compound 7: Berberine ¹HNMR (400 MHz, CD₃OD): δ 9.77(1H,s, H-8), 8.68(1H,s, H-13), 8.11(1H, d, *J*=8.0 Hz, H-12), 8.00(1H, d, *J*=8.0 Hz, H-11), 7.64(1H,s, H-1), 6.95(1H,s,H-4), 6.10(2H,s, -OCH₂O-), 4.94(2H,t,*J*=3.0 Hz, H-6), 4.20 (3H,s, 9-OCH₃), 4.10(3H,s, 10-OCH₃), 3.27(2H, t, *J*=3.0 Hz H-5); ¹³CNMR(100 MHz, CD₃OD): δ152.1(C-10), 152.0(C-3), 149.8(C-2), 146.3(C-9), 145.7(C-8), 139.5(C-13a), 135.1(C-12a), 131.87(C-4a), 128.0(C-11), 124.5(C-12), 123.2(C-8a), 121.8(C-13b), 121.4(C-13), 109.3(C-4), 106.5(C-1), 103.6(-OCH₂O-), 62.5(9-OCH₃), 57.6(10-OCH₃), 57.1(C-6), 28.1(C-5).

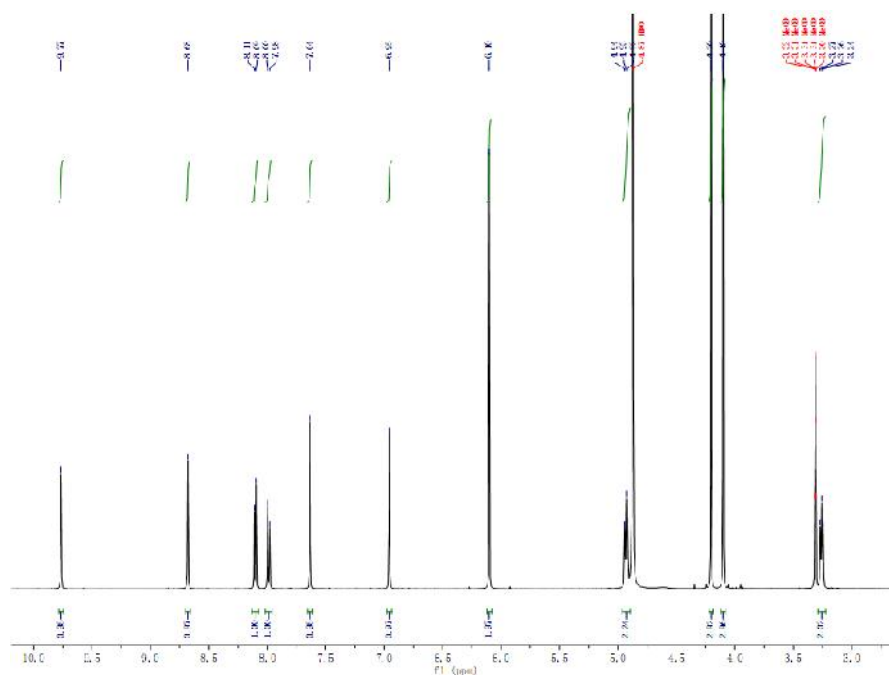


Fig.S20 ¹HNMR spectra of Berberine

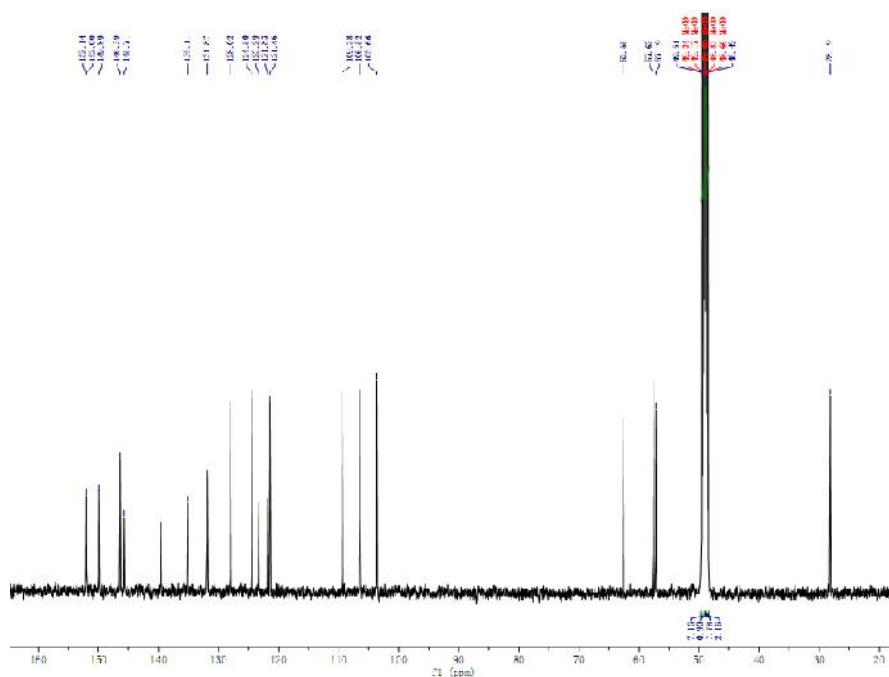


Fig.S21 ¹³CNMR spectra of Berberine

