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Preparation and stability of chebulagic acid and chebulinic acid from *Terminalia chebula* and their biological activity

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Abstract: Chebulagic acid and chebulinic acid are the two tannin compounds with the highest content in *Terminalia chebula,* they were separated by ODS column eluted with 20% methanol and 35% methanol, respectively. The compounds were identified by comparing the data of ^{1}H NMR and ^{13}C NMR with the literature; HPLC method was used to investigate the stable storage conditions of chebulagic acid and chebulinic acid; lipopolysaccharide (LPS) induced in vivo inflammation model and RAW264.7 macrophage *in vitro* inflammatory model to evaluate the anti-inflammatory activities of chebulagic acid and chebulinic acid. HepG2 cells were used to study the anti-hepatoma activities of two compounds. The structure of the isolated chebulagic acid and chebulinic acid was consistent with that reported in the literature. They could be dissolved in different concentrations of acetonitrile and stored in cold storage. Both of them have good anti-inflammatory and anti-hepatoma activities.

Keywords: *Terminalia chebula,* chebulagic acid, chebulinic acid, stability, anti-inflammatory, anti-hepatoma.

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INTRODUCTION

Terminalia chebula is native to India. Nowadays, it is also cultivated in Yunnan, Guangxi, Guangdong provinces of China. *Terminalia chebula* was included in the every edition of Chinese Pharmacopoeia as an astringent. It is recorded as the dried and mature fruit of *Terminalia chebula Retz.* and *Terminalia chebula Retz.* var. *tomentella* Kurt. in the 2020 edition. It is bitter, sour, astringent, flat in nature, belongs to the lung and large intestine meridian and has the effects of astringent intestine, relieving diarrhea, astringent lung, relieving cough and reducing fire (Chinese Pharmacopoeia Commission, 2020). Modern chemical research shows that the main chemical components of *Terminalia chebula* are polyphenols, tannin, gallic acyl glucose and triterpenoid acids (Nigam *et al.*, 2020). *Terminalia chebula* has strong antioxidant (Randhawa *et al.*, 2021), anti-inflammatory (Zhang *et al.*, 2016). anticancer (Shendge *et al.*, 2020). and inhibition α-Glycosidase (Pingali *et al.*, 2020). activity, modern pharmacological studies had proved that polyphenols and tannins are the main active components (Zhang *et al.*, 2021). Among, chebulagic acid (CGA) and chebulinic acid (CNA) are the representative compounds of *Terminalia chebula*. However, the preparation methods of CGA and CNA reported in the literature are various column chromatography, high-speed countercurrent chromatography and high performance liquid preparation chromatography (Zou *et al.*, 2016; Yang *et al.*, 2019), which has the disadvantages of high cost, cumbersome steps and low yield. In the early stage of this study, the preparation methods of the two compounds were studied, but in the process of study, it was found that the two compounds were unstable in high concentration methanol. Through mass spectrometry analysis, it was found that they had condensation reaction with methanol. Therefore, based on the preparation of CGA and CNA from *Terminalia chebula* and the identification of their structures through literature comparison, this study further carried out a more systematic study on the storage methods and use conditions of the two compounds, namely, stability and explored their anti-inflammatory and anti-hepatoma activities, providing material and theoretical basis for the quality standard and clinical application of *Terminalia chebula*.

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MATERIALS AND METHODS

Materials

Terminalia chebula, a medicinal material produced in Yunnan Province (China); Lipopolysaccharide (purity ≥ 97%, Dalian Meilun Biotechnology Co., Ltd, Dalian, China); Dexamethasone (0.75 mg/pill, 2009221, Suicheng Pharmaceutical Co., Ltd, Tianjin, China); Dexamethasone (purity ≥ 99%, 7211057, cell level, Beijing Solarbio Technology Co., Ltd, Beijing, China); T-AOC test kit (BC1315, Solarbio Biotechnology Co., Ltd, Beijing, China); Mouse TNF-α, SOD, MDA, IL-1β, IL-6 elisa kits (F2132-A, F2040-A, F2163-A, F2389-A, F9264-A,

Shanghai Kexing Reagent Co., Ltd, Shanghai, China); Reference materials of CGA and CNA (PRF8112302, PRF8071201, Chengdu purifa Technology Development Co., Ltd, Chengdu, China); Mouse monocyte macrophage cell RAW264.7 and human hepatoma cells HepG2 (Shanghai saibaikang Biotechnology Co., Ltd, Shanghai, China); Nitric oxide detection kit (07082121116, Shanghai biyuntian Biotechnology Co., Ltd, Shanghai, China).

Shimadzu-16 high performance liquid chromatography system (Shimadzu company, Japan); NIB-100 inverted microscope (Ningbo Yongxin Optical Co., Ltd, Ningbo, China); Multiskan-MK3 microplate reader (Thermo Fisher Scientific China Co., Ltd, Shanghai, China).

Clean grade KM mice, half male and half female (20±2 g), purchased from Liaoning Changsheng Biotechnology Co., Ltd., (Benxi, China) and the animal production license number is SCXK (辽) 2020-0001). The mice were raised in the environment of (25±3) ºC and relative humidity of (55±10)%, alternating day and night every 12 hours, during which they were free to eat and drink water, and were raised adaptively for one week.

Extraction and isolation

The *Terminalia chebula* were crushed after stone of being removed and sieved through 65 meshes. 20g *Terminalia chebula* powder was added with 200ml of 70% ethanol for ultrasonic extraction for 30min. The extract was centrifuged at 3000 rpm for 10min and the supernatant was taken to recover ethanol. The residue was re dissolved with 20ml of 20% methanol and centrifuged at 10000 rpm for 10 min. The supernatant was separated by ODS open column. 20 column volumes were eluted with 20% methanol and 10 column volumes were eluted with 35% methanol. The eluent was collected and examined by HPLC. The parts containing CGA or CNA were combined respectively. The solvent was recovered, CGA was thermally dissolved in water, CNA was thermally dissolved in 30% methanol and placed them at 4ºC until the solid was precipitated. The compounds were dissolved in CD_3OD after drying and ^1H NMR, ^{13}C NMR and MS were determined. This NMR work employed Agilent 400MR and MS work employed a 6550 Q-TOF/MS system to obtain the MS data using the electrospray ionization source in negative-ion mode. Further more, this work optimized the MS conditions as given below: capillary voltage, 3500 V; the drying gas and sheath gas were high purity nitrogen; drying gas flow rate, 11 L/min; nozzle voltage, 500 V; drying gas temperature, 150 ·; atomizer pressure, 25 psi; sheath gas flow rate, 10 L/min; sheath gas temperature, 350ºC; fragmentation voltage, 125 V; and the mass scanning range was 50-1500 m/z.

Investigation on the stability of compounds

CGA or CNA were placed in different environments, the peak area was detected by HPLC after 0, 2, 4, 6, 8 and 10 hours and RSD was calculated to explore the stable storage environment of CGA and CNA.

Chromatographic conditions

Chromatographic column: dikma platisil (250 mm × 4.6 mm, 5μm); Mobile phase of CGA: phase A was water: methanol: formic acid (90: 10: 0.1 V/V/V); phase B was acetonitrile, elution gradient: 0~2 min, 5%→13%B; 2~20 min, 13% B; Mobile phase of CNA: phase A was water: methanol: formic acid (70: 30: 0.1 V/V/V); phase B was acetonitrile, elution gradient: 0~5 min, 5%→11%B; 5~20 min, 11%B; Column temperature: 25ºC; Flow rate: 1.0 ml/min; Injection volume: 5μl; Detection wavelength: 278 nm (CGA), 280 nm (CNA).

CGA and CNA were dissolved at a mass concentration of 0.1mg/ml in acetonitrile respectively and injected continuously for 6 times under the above chromatographic conditions. The peak area RSD was calculated and the precision of the instrument was investigated.

Investigation of Solvent Types

CGA or CNA was dissolved in different solvents (30% methanol, 50% methanol, 70% methanol, 30% acetonitrile, 50% acetonitrile, 70% acetonitrile and absolute ethanol) to prepare solutions with a concentration of about 0.1mg/ml, place them at room temperature. Detect the peak area by HPLC after 0, 2, 4, 6, 8 and 10 hours, calculate RSD, and explore the stable storage solvent of CGA and CNA.

Investigation of Temperature

CGA or CNA was dissolved in 50% acetonitrile to prepare solutions with a concentration of about 0.1 mg/ml, place them at different temperatures (-20ºC, 4ºC, 25ºC, 80ºC, 90ºC and 100ºC). Detect the peak area by HPLC after 0, 2, 4, 6, 8 and 10 hours, calculate RSD, and explore the stable storage temperature of CGA and CNA.

Investigation of Mass Concentration

CGA or CNA was dissolved in 50% acetonitrile, prepare solutions with concentrations of about 0.01, 0.1 and 1 mg/ml respectively, place them at room temperature. Detect the peak area by HPLC after 0, 2, 4, 6, 8 and 10 hours, calculate RSD, and explore the stable storage mass concentration of CGA and CNA.

Investigation of pH Value

CGA or CNA was dissolved in 50% acetonitrile, add acetic acid or triethylamine, adjust the pH value to 3, 4, 5, 6, 7 and 8 respectively, make solutions with a concentration of about 0.1mg/ml, place them at room temperature. Detect the peak area by HPLC after 0, 2, 4, 6, 8 and 10 hours, calculate RSD and explore the stable storage pH value of CGA and CNA.

Stability prediction
Experimental data at 80, 90 and 100ºC of temperature investigation were took, the stability of CGA and CNA were predicted by classical constant temperature method (Zhang *et al.*, 2012). The reaction rate constants at different temperatures were obtained by time regression with the logarithm of each concentration. According to Arrhenius exponential law, logK=lgA-E/2.303TR, linear regression is carried out for 1/T with logK to calculate the validity period and half-life of CGA and CNA under room temperature (25ºC), cold storage (4ºC) and freezing (-20ºC).

Study on anti-inflammatory activity
Animal in vivo experiment
Mice were randomly divided into 9 groups: blank group (BG), model group (MG), dexamethasone group (DEX), high dose groups of CGA (HCGA), medium dose groups of CGA (MCGA), low dose groups of CGA (LCGA), high dose groups of CNA (HCNA), medium dose groups of CNA (MCNA), low dose groups of CNA (LCNA), with 10 mice in each group. The dosages of CGA and CNA were 65, 130 and 260mg/kg, dexamethasone was 0.4 mg/kg. (The medium dosage was calculated according to the maximum daily oral dose of adults recorded in Chinese Pharmacopoeia 2020 Edition or the corresponding instructions). All of mice were gavaged with 0.4 ml/kg of corresponding drug solution, except that blank group and model group were gavaged with the same dose of water, once a day for a total of three days. One hour after administration on the third day, except for the blank group, mice in each group were intraperitoneally injected with LPS with a concentration of 1mg/ml to prepare the mouse sepsis model and the dose was 0.2ml/20g. After 12 hours of modeling, mice in each group took eyeballs and collected blood, routinely separated serum, and detected TNF-α, IL-6, IL-1β, SOD and MDA in serum by ELISA; The liver and lung tissues were dissected, the same part of every mice were took and fixed with 4% formaldehyde, stained and sliced by HE method, and the pathological changes were observed.

Cell in vitro experiment
RAW 264.7 cells were cultured in DMEM complete medium (10% FBS, 1% penicillin, streptomycin) at 37ºC and 5% CO_2. The cells in logarithmic growth phase were selected and the density was 2×10^5 /ml were evenly inoculated on 96 well plates, 100μl per well. After 24 hours of adherent culture, the blank group was added with complete medium and the model group was added with a final concentration of 1μg/ml LPS in complete medium; the final concentration of 50μmol/L was added in the administration group on the basis of the model group, and the positive control was dexamethasone (DEX). After 24 hours of treatment, the NO release of each group was measured according to the standard operation of Griess reagent. The viability of cells in the same batch was determined by CCK-8 method. Three multiple holes were set in all experiments.

Study on anti-hepatoma activity
The cultivation method of HepG2 cells is the same as above. The cells in logarithmic growth phase were selected and the density was 1×10^5 /ml were evenly inoculated on 96 well plates, 100μl per well. In addition, 3 blank holes were set and only 100μl of culture medium was added, without cells. After 24 hours of adherent culture, the blank group and control group were added with 100μl of culture medium, CGA group and CNA group were added with 100μl of different concentrations of culture medium containing medicinal (800, 400, 200, 100, 50, 25 μmol/L). Three multiple holes were set in all experiments. After continuous cultivation for 24, 48 and 72 hours, the culture medium was discard, 100μl of 10% CCK-8 solution were added and then the absorbance (OD) value was meassured at 450 nm with a micro plate reader after 2 hours of cultivation in the incubator away from light.

Ethical approval
The animal-related experiments in this study have been approved by the Laboratory Animal Ethics Committee of Liaoning University of Traditional Chinese Medicine, with the approval No.2019YS (DW)-010-01.

STATISTICAL ANALYSIS

SPSS 17.0 software was used for one-way ANOVA and LSD test to analyze the experimental data. The results were expressed as the mean ± standard deviation. $P<0.05$ was accepted as the significant difference.

RESULTS

Isolation and Identification
The 520 mg of CGA was obtained as a white powder and yield was 2.6%. The protonated molecule was proved to be m/z 953.0954 $[M-H]^-$ by MS and the molecular formula was proved to be $C_{41}H_{30}O_{27}$, the structure was consistent with that of CGA reported in the literature (fig. 1) (Zou *et al.*, 2016; Yang *et al.*, 2019; Luo *et al.*, 2012). The 600 mg of CNA was obtained as a white crystal and yield was 3.0%. The protonated molecule was proved to be m/z 955.1126 $[M-H]^-$ by MS, and the molecular formula was proved to be $C_{41}H_{32}O_{27}$, the structure was consistent with that of CNA reported in the literature (fig. 1) (Zou *et al.*, 2016; Yang *et al.*, 2019). The data of ^1H NMR and ^{13}C NMR for CGA and CNA were showed in table 1. The spectra of MS were shown in fig. 2.

Stability
Univariate study results
The precision of the instrument has been verified. The RSD of CGA peak area was 0.25% and CNA was 0.33%.

Both CGA and CNA achieved baseline separation, with a separation degree greater than 2 and a theoretical number of trays greater than 8000 (fig. 3). The investigation results of different solvents showed that within 10 h, CGA and CNA were stable in different concentrations of acetonitrile, stable in ethanol and but unstable in methanol, and the stability reduced with the increase of methanol content in solution. The investigation results of different temperatures showed that within 10 h, CGA and CNA were stable at room temperature and lower, but they were very unstable at high temperature (more than 80℃); The results of different mass concentrations showed that the concentration had no effect on the stability of CGA and CNA; The investigation results of different pH values show that CGA and CNA are stable under acidic conditions and unstable under alkaline conditions. (table 1 and fig. 4)

The prediction results of classical thermostatic method

The reaction rate constants at different temperatures were obtained by time regression with the logarithm of each concentration (table 2). The degradation reaction of CGA and CNA accorded with the first-order kinetic reaction. According to Arrhenius exponential law, logK=lgA-E/2.303TR, linear regression was carried out for 1/T with logK to calculate the validity period and half-life of CGA and CNA under room temperature (25°C), cold storage (4°C) and freezing (-20°C) (table 3). The results showed that when CGA and CNA were dissolved in 50% acetonitrile, they could be stored at room temperature (25°C) in a short time (CGA: 192.14 d, CNA: 19.53 d), and stored in cold storage (4°C) for a long time (CGA: 8192.70 d, CNA: 356.92 d) and freezing (-20°C or lower) is not required.

Results of anti-inflammatory activity

The determination of anti-oxidation and inflammatory factors showed that both CGA and CNA had certain anti-inflammatory effects (table 4). Overall, the effect of high dose is not significantly better than that of low dose.

The liver tissue in the model group had severe bleeding and inflammatory cell infiltration; Severe bleeding of lung tissue, thickening of alveolar septum and infiltration of inflammatory cells; The situation of each treatment group was improved (fig. 5).

CCK-8 test shows that the concentration of CGA and CNA were less than 100μmol/L had no significant cytotoxicity, and at 50μmol/L had better anti-inflammatory effect, the release of NO was significantly lower than that of the model group, KG: 2.33±1.15, MG: 36.90±1.58, DEX: 29.10±3.01, CGA: 25.73±3.01, CNA: 24.13±3.61 (fig. 6).

Results of anti-hepatoma activity

The results showed that the CGA and CNA have certain anti hepatoma activity and the IC50 after 24, 48 and 72 hours of CGA were 198.85, 163.06 and 96.81 μmol/L respectively, the IC50 after 24, 48 and 72 hours of CNA were 502.97, 268.32 and 156.40 μmol/L respectively.

DISCUSSIONS

Compared with the existing methods (Zou *et al.*, 2016; Yang *et al.*, 2019), the method for preparing CGA and CNA in this study saves time and effort, uses simple device and has high yield. According to the results of stability investigation, the two compounds are stable in different concentrations of acetonitrile and methanol with concentrations below 30%. Considering the stability of the compounds and the solvent effect in chromatographic separation as the reference substance, it is more appropriate to choose different concentrations of acetonitrile as the solvent, if methanol must be used, the recommended concentration is less than 30%. CGA and CNA are unstable under neutral and alkaline conditions, which may be due to the presence of multiple phenolic hydroxyl groups in the molecular structure of CGA and CNA. Therefore, it is more appropriate for CGA and CNA to be dissolved in weak acid solution. when CGA and CNA were dissolved in 50% acetonitrile, they could be stored at room temperature (25°C) in a short time (CGA: 192.14 d, CNA: 19.53 d) and stored in cold storage (4°C) for a long time (CGA: 8192.70 d, CNA: 356.92 d) and freezing (-20°C or lower) is not required.

Lipopolysaccharide (LPS) is the surface antigen of Gram-negative bacteria. As a strong and effective stimulator of the immune system, the host can start the immune defense response in the early stage of infection. It is often used as a modeling drug for inflammatory model (Khan *et al.*, 2021; Liu *et al.*, 2022). LPS can cause inflammatory reaction and oxidative stress reaction and cause damage to multiple organs. The liver is a detoxification organ, and LPS is the most likely to cause liver injury (Pervin *et al.*, 2018; Li *et al.*, 2021); The occurrence of lung injury will make the antioxidant imbalance in the body and the uncontrolled oxidative stress and inflammatory response will further lead to the development of lung injury. In addition, lung injury will also aggravate the infiltration of inflammatory cells (Ye and Liu, 2020). At the same time, in the traditional theory of traditional Chinese medicine, *Terminalia chebula* is mainly used to treat lung diseases. Therefore, this study investigated anti inflammatory effect and the liver and lung protective effects of CGA and CNA on LPS induced inflammatory response. And study the anti-hepatoma activity of CGA and CNA. The results showed that CGA and CNA have good biological activity. This experiment not only verified the activity of CGA and CNA, but also explored their storage conditions, providing a theoretical basis for their development as first-line anti-inflammatory or anticancer drugs in clinical practice.

Table 1: NMR Spectral data for CGA and CNA (400 MHz, CD_3OD).

Position	CGA		CNA		
	^{1}H	^{13}C	^{1}H	^{13}C	Type
1	6.50 (1H, d, *J*=1.3 Hz)	92.59	6.49 (1H, d, *J*=2.8 Hz)	92.95	-OCH-
2	5.39 (1H, brs)	71.16	5.43 (1H, brs)	71.97	-OCH-
3	5.82 (1H, brs)	62.47	6.23 (1H, brs)	62.94	-OCH-
4	5.22 (1H, d, *J*=3.5 Hz)	67.06	5.04 (1H, d, *J*=3.2 Hz)	69.59	-OCH-
5	4.81 (1H, d, *J*=5.4 Hz)	74.33	4.72 (1H, m)	76.19	-OCH-
6	4.37(1H, dd, J=10.5, 7.6 Hz), 4.92(1H, d, J=10.5 Hz)	64.79	4.62(1H, dd, J=10.7, 6.3 Hz), 4.80(1H, dd, J=10.7, 7.7 Hz)	65.34	-OCH$_2$-
1'	/	170.83	/	170.82	>C=O
2'	4.82 (1H, s)	66.85	4.82 (1H, d, *J*=5.4 Hz)	67.19	-CH-OH
3'	5.05 (1H, dd, *J*=7.2, 1.6 Hz)	41.79	5.09 (1H, dd, *J*=7.1, 1.6 Hz)	41.83	-CH-
4'	3.80 (1H, ddd, *J*=11.6, 3.8, 1.6 Hz)	40.06	3.87 (1H, ddd, *J*=10.5, 4.8, 1.6 Hz)	40.14	-CH-
5'	2.19 (1H, dd, *J*=17.0, 3.8 Hz), 2.11 (1H, dd, *J*=17.0, 11.6 Hz)	30.55	2.23 (1H, d, *J*=8.0 Hz), 2.25 (1H, d, *J*=2.2 Hz)	30.80	-CH2-
6'	/	175.05	/	174.99	>C=O
7'	/	174.48	/	174.64	>C=O
1"	/	116.29	/	119.14	=C
2"	7.48 (1H, s)	119.09	7.51 (1H, s)	117.70	=C
3"	/	117.69	/	147.50	=C
4"	/	147.46	/	140.52	=C
5"	/	140.45	/	141.55	=C
6"	/	141.47	/	116.22	=C
7"	/	166.47	/	166.15	>C=O
1'''	/	120.15	/	119.86, 120.09, 120.78	=C
2'''	7.07 (2H, s)	110.98	7.10, 7.17, 6.98 (2H, s)	110.30, 110.76, 110.82	=C
3'''	/	146.62	/	146.49, 146.65, 146.75	=C
4'''	/	140.93	/	140.16, 140.86, 140.93	=C
5'''	/	146.62	/	146.49, 146.65, 146.75	=C
6'''	7.07 (2H, s)	110.98	7.10, 7.17, 6.98 (2H, s)	110.30, 110.76, 110.82	=C
7'''	/	166.34	/	166.19, 166.34, 167.95	>C=O
1''''	/	116.03	/	/	=C
2''''	6.84 (1H, s)	125.65	/	/	=C
3''''	/	108.26	/	/	=C
4''''	/	146.22	/	/	=C
5''''	/	137.63	/	/	=C
6''''	/	145.41	/	/	=C
7''''	/	170.20	/	/	>C=O
1'''''	/	117.72	/	/	=C
2'''''	6.64 (1H, s)	124.59	/	/	=C
3'''''	/	110.52	/	/	=C
4'''''	/	145.67	/	/	=C
5'''''	/	138.74	/	/	=C
6'''''	/	145.62	/	/	=C
7'''''	/	167.56	/	/	>C=O

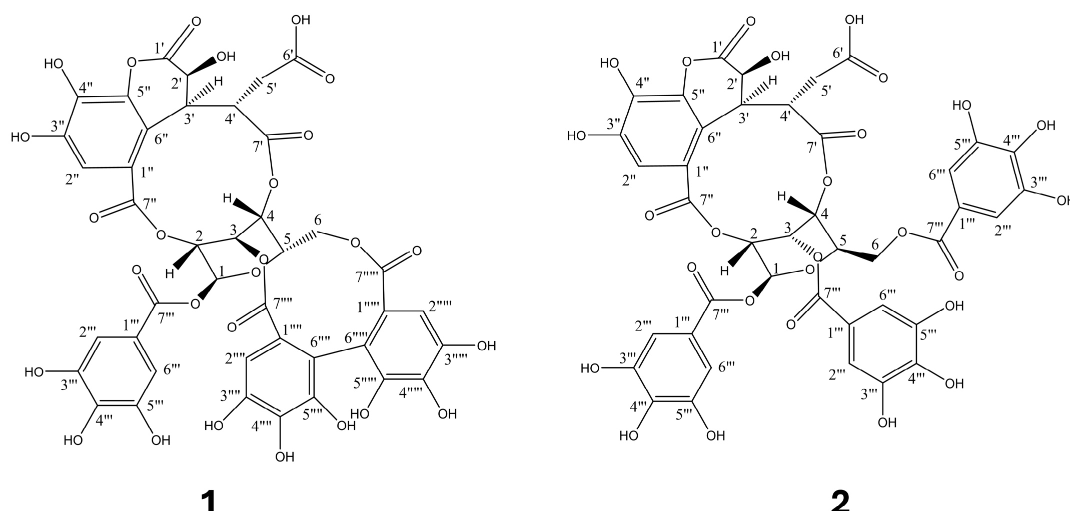
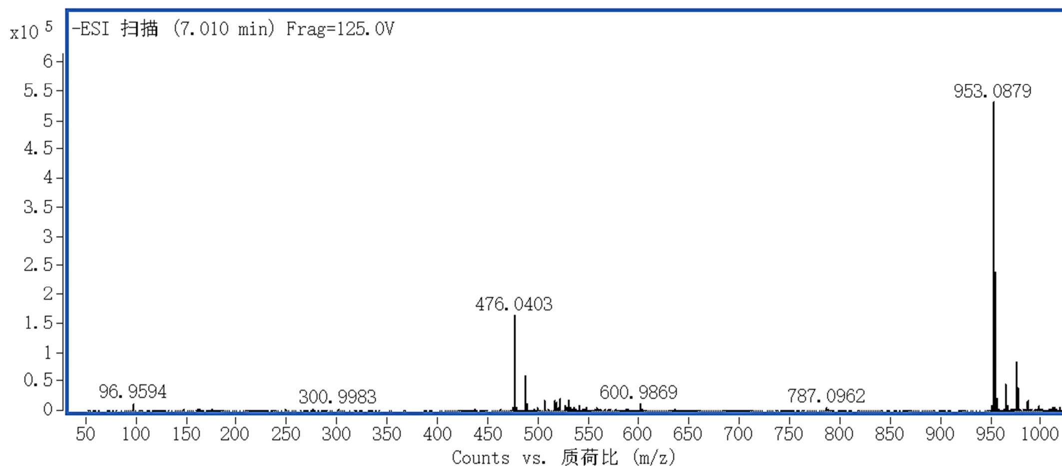


Fig. 1: Compounds structure, CGA; (2) CNA

Table 2: stability investigation results of CGA and CNA.

Project		RSD/%	
		CGA	CNA
solvent	30% methanol	0.70	0.82
	50% methanol	1.49	1.55
	70% methanol	1.45	1.55
	100% methanol	2.89	1.79
	30% acetonitrile	0.33	0.23
	50% acetonitrile	0.34	0.15
	70% acetonitrile	0.17	0.15
	100% acetonitrile	0.32	0.33
	100% ethanol	0.45	0.16
temperature	-20°C	0.44	0.91
	4°C	0.22	0.11
	25°C	0.34	0.15
	80°C	17.08	29.06
	90°C	53.66	60.71
	100°C	102.47	112.66
concentration	Low concentration	0.27	0.67
	Medium concentration	0.34	0.15
	High concentration	0.11	0.29
pH value	pH3	0.59	0.21
	pH4	0.29	0.30
	pH5	0.36	0.31
	pH6	0.22	0.49
	pH7	189.80	165.48
	pH8	211.93	155.81



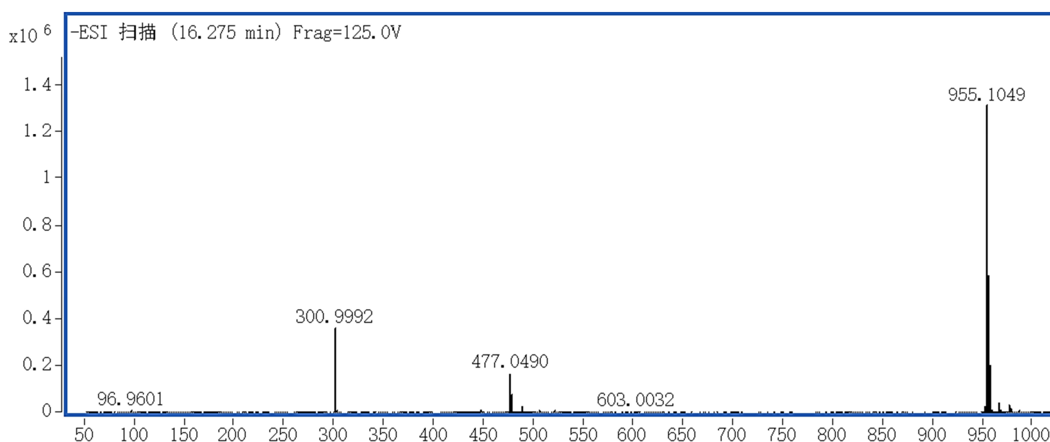


Fig. 2: Mass spectrum, A: CGA; B: CNA

Table 3: K, 1/T and lgK of CGA and CNA at different temperatures

Compounds	T(ºF)	Reaction equation	K	1/T	lgK
CGA	353	lgC=-0.0208t+2.0113	0.0479024	0.002832861	-1.319642727
	363	lgC=-0.0801t+2.0714	0.1844703	0.002754821	-0.734073546
	373	lgC=-0.1948t+2.0770	0.4486244	0.002680965	-0.348117109
CNA	353	lgC=-0.0375t+2.0356	0.0863625	0.002832861	-1.063674794
	363	lgC=-0.0980t+2.0986	0.2256940	0.002754821	-0.646479986
	373	lgC=-0.2123t+2.0436	0.4889269	0.002680965	-0.310756068

Table 4: The validity period and half-life of CGA and CAN

Compounds	T(ºC)	validity period (d)	half-life (d)
CGA	25	192.14	1263.31
	4	8192.70	53866.63
	-20	1280467.32	8419011.90
CNA	25	19.53	128.38
	4	356.92	2346.74
	-20	17838.11	117284.70

Table 5: Determination of antioxidant indexes and inflammatory factors

Group	T-AOC (μmol/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	SOD (pg/mL)	MDA (nmol/mL)
BG	1.08±0.07	108.51±3.62	122.11±3.79	625.78±15.83	193.58±14.70	4.19±0.21
MG	0.69±0.12#	78.44±4.01#	253.75±59.58#	750.36±25.47#	159.85±3.64#	8.00±0.33#
DEX	0.89±0.06	122.10±7.42*	130.15±3.57*	692.87±14.82*	188.36±8.12*	4.20±0.11*
HCGA	1.03±0.18*	125.94±3.79*	127.97±3.23*	673.91±8.53*	290.00±29.82*	5.89±1.43*
MCGA	0.83±0.11	126.88±3.83*	136.52±10.38*	645.74±21.35*	246.68±9.03#*	6.33±0.98*
LCGA	0.83±0.19	120.44±6.14*	148.63±41.37*	673.32±23.18*	248.77±13.31*	6.47±0.51*
HCNA	0.90±0.15	121.07±3.50*	124.30±5.86*	658.19±29.45*	250.47±10.67*	4.51±0.96*
MCNA	0.85±0.15	117.25±8.09*	121.49±8.16*	646.42±30.75*	231.90±20.31*	4.53±1.16*
LCNA	0.77±0.16	123.15±3.50*	142.62±37.16*	662.20±19.89*	230.16±22.00*	5.51±0.14*

Note: Compared with the BG, #p<0.05; Compared with the MG, *P<0.05.

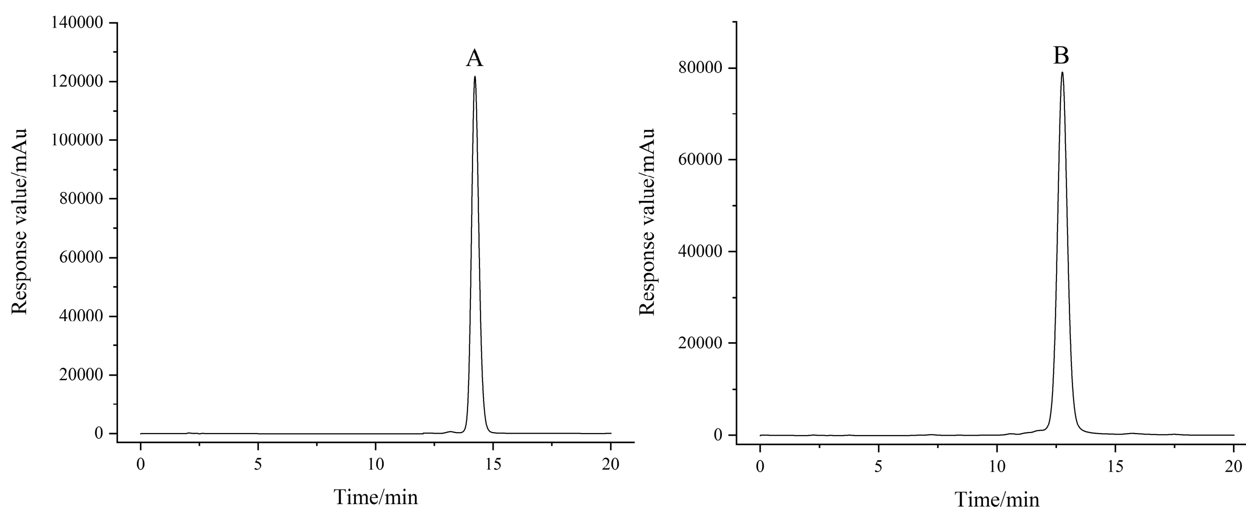


Fig. 3: System adaptability test of CGA (A) and CNA(B)

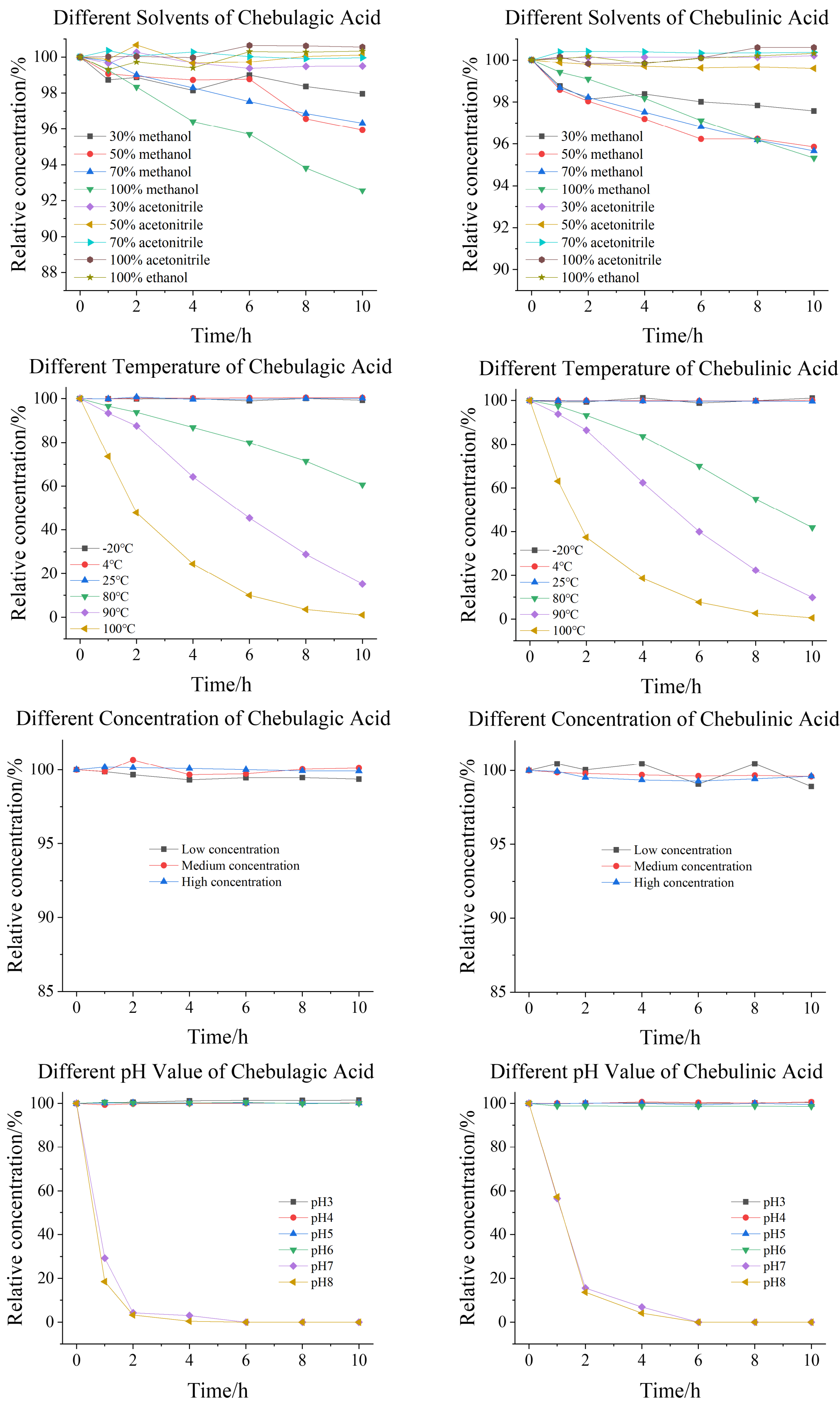


Fig. 4: stability investigation results of CGA and CNA

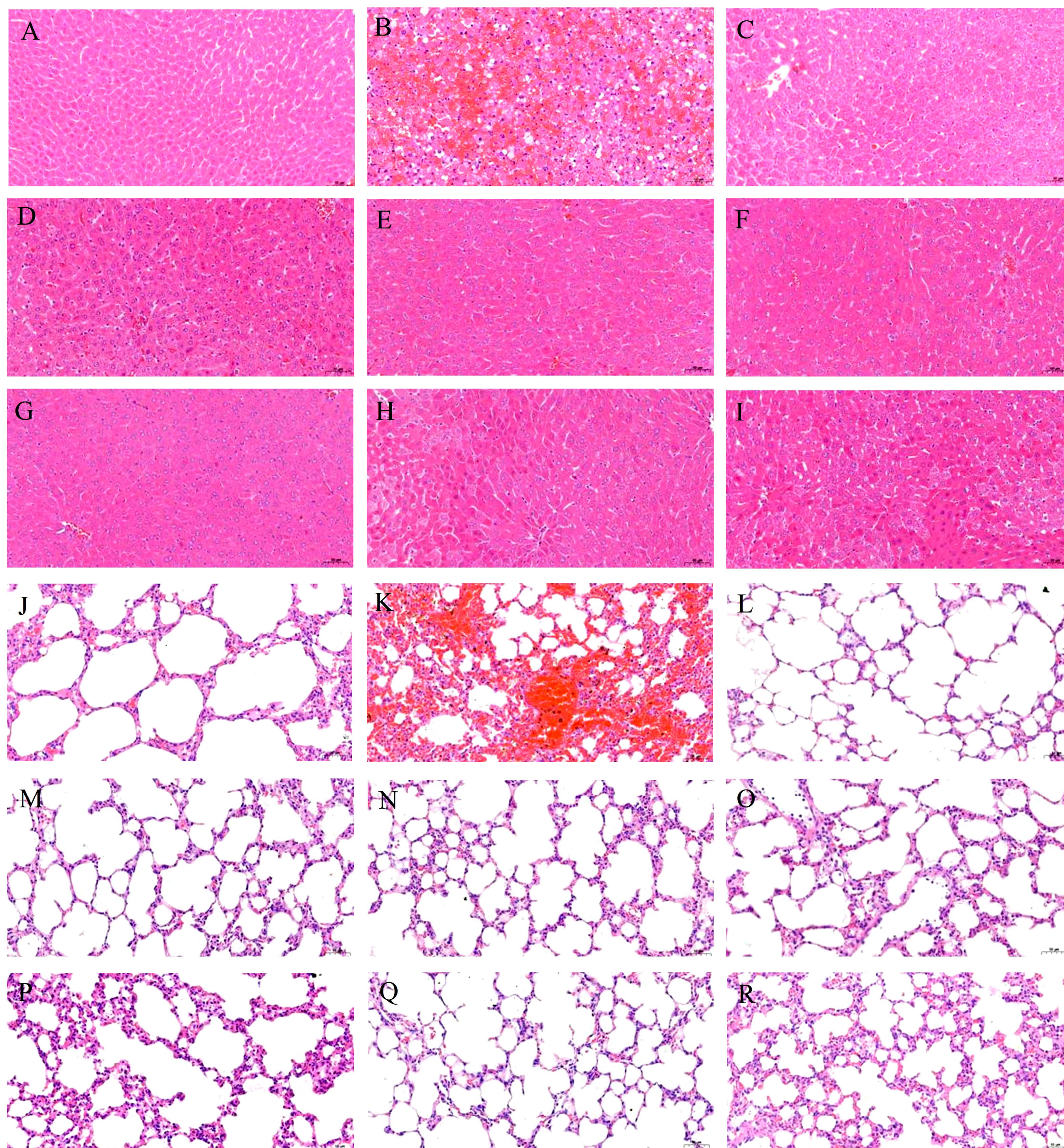
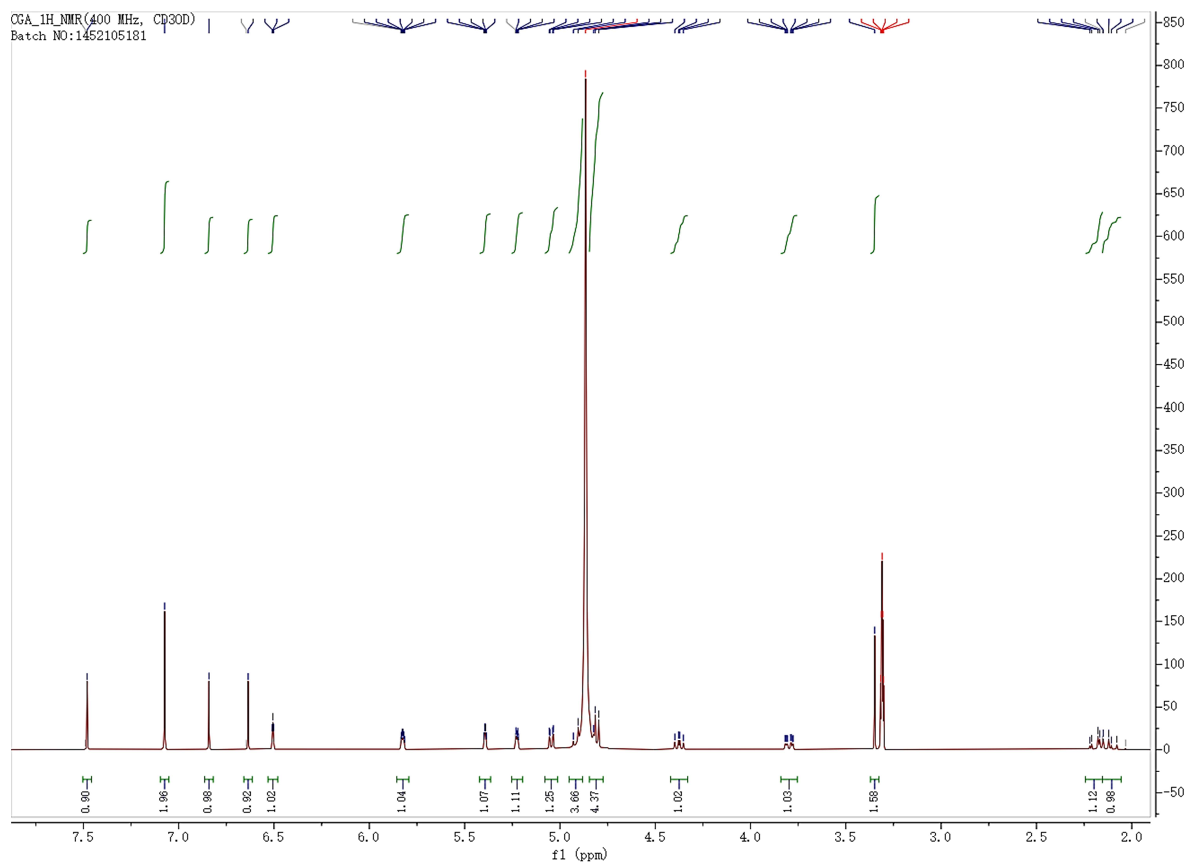
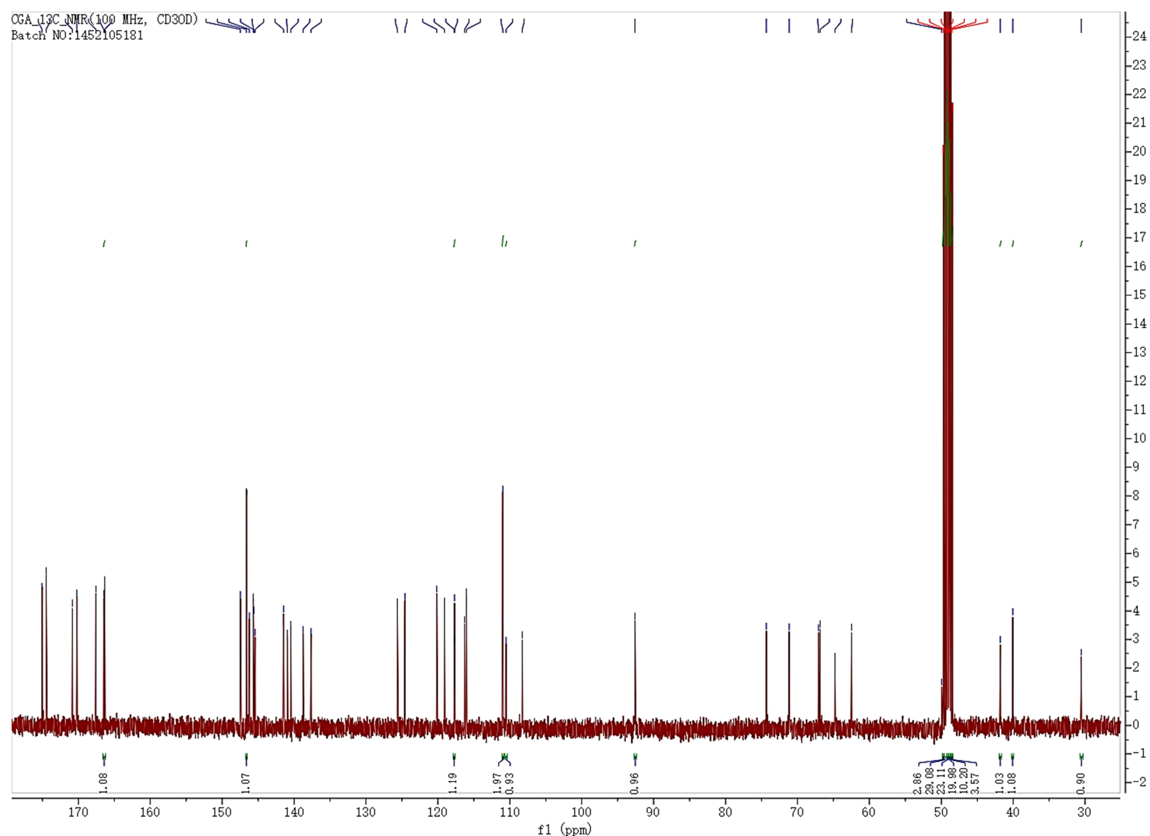


Fig. 5: Pathological sections of liver and lung of mice in each group (200×), A. liver of BG; B. liver of MG; C. liver of DEX; D. liver of HCGA; E. liver of MCGA; F. liver of LCGA; G. liver of HCNA; H. liver of MCNA; I. liver of LCNA; J. lung of BG; K. lung of MG; L. lung of DEX; M. lung of HCGA; N. lung of MCGA; O. lung of LCGA; P. lung of HCNA; Q. lung of MCNA; R. lung of LCNA;





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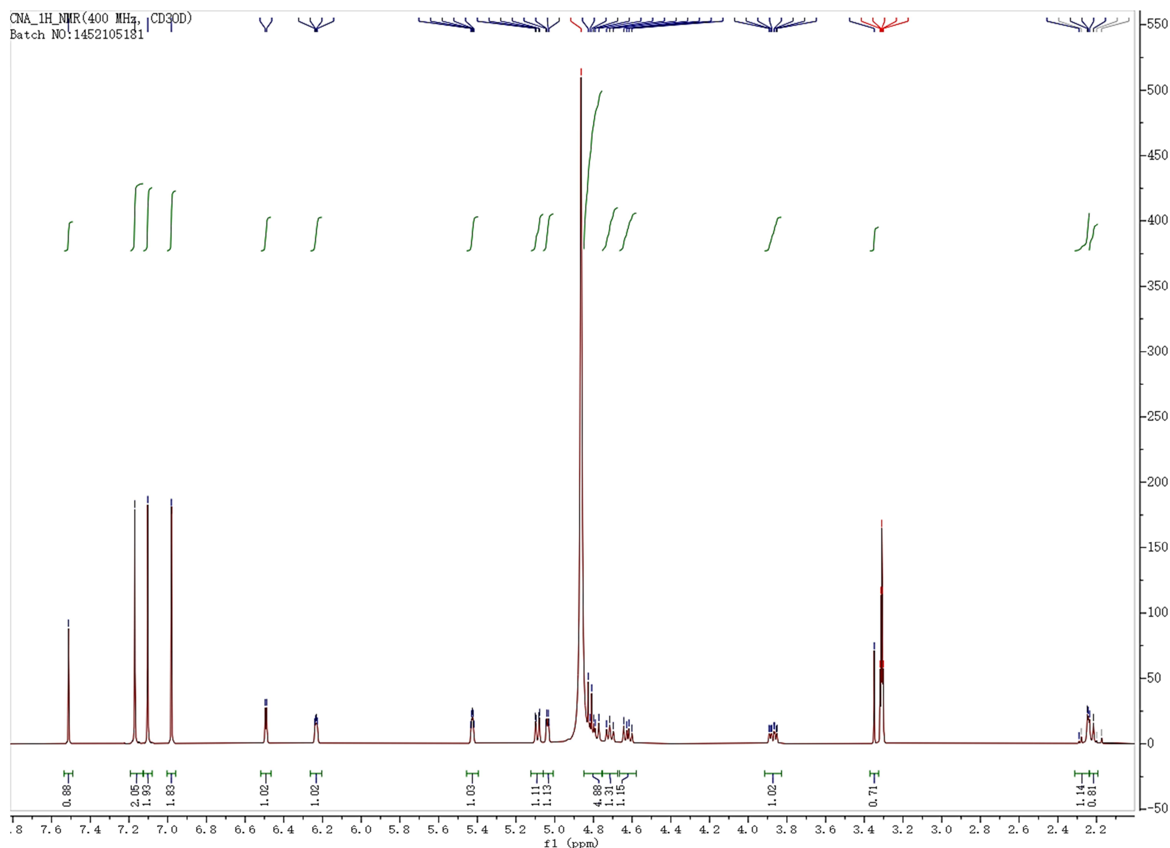

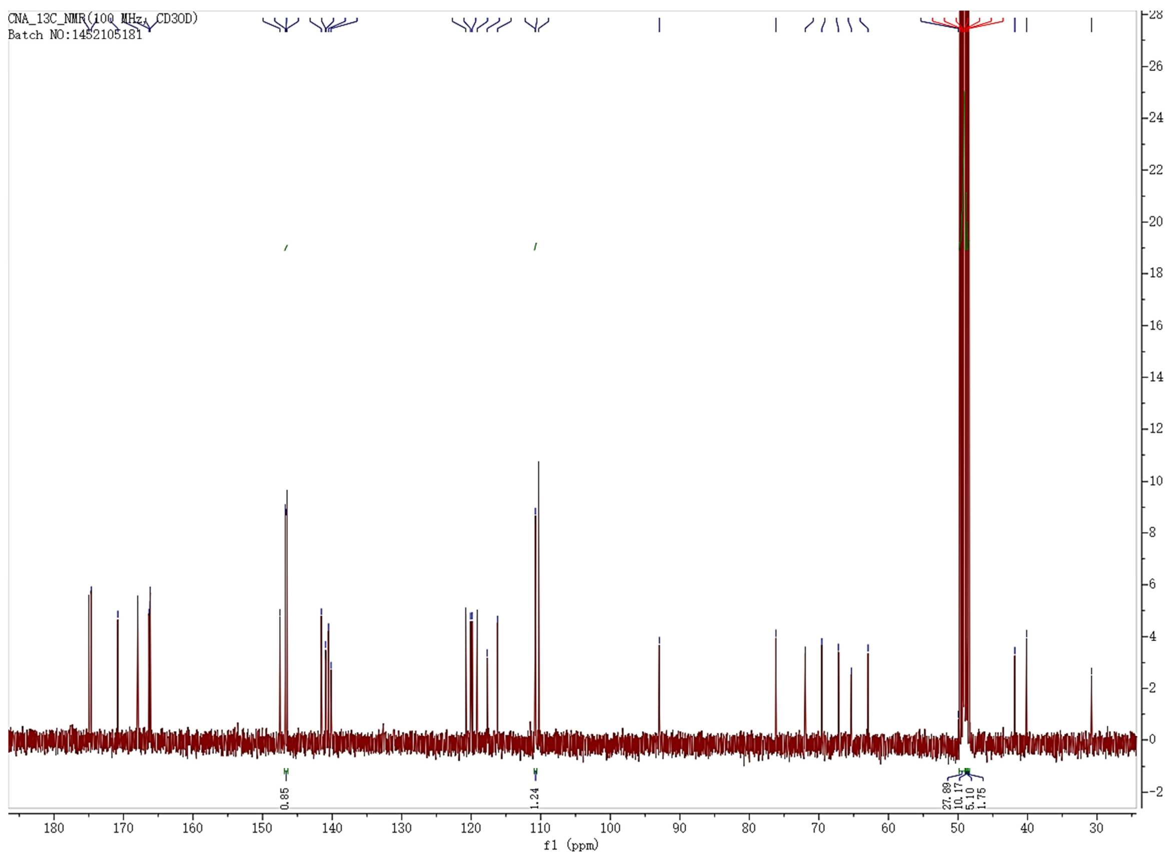

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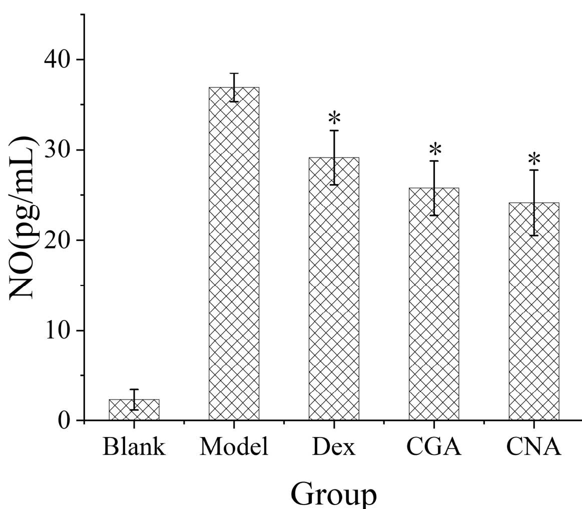

Fig. 6: No release in each group

CONCLUSION

To sum up, CGA and CNA belong to tannin compounds, different concentrations of acetonitrile or methanol lower than 30% can be selected as the solvent. At the same time, they also have good anti-inflammatory and anti-hepatoma activity. This study can provide a certain basis for the quality standard and clinical application of *Terminalia chebula*.

CONFLICT OF INTEREST

No conflict of interest is associated with this work.

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