

## *Lactobacillus casei* Qian Enhances the Preventive Effect of Geniposide on HCl/Ethanol Induced Gastric Injury in Mice

QIAN Yu<sup>1</sup>, SUO Huayi<sup>2</sup>, YI Ruokun<sup>1</sup>, LI Guijie<sup>1</sup>, ZHAO Xin<sup>1,\*</sup>

(1. Chongqing Engineering Laboratory for Research and Development of Functional Food, Chongqing Engineering Research Center of Functional Food, Chongqing Collaborative Innovation Center for Functional Food, College of Biological and Chemical Engineering, Chongqing University of Education, Chongqing 400067, China;

2. College of Food Science, Southwest University, Chongqing 400715, China)

**Abstract:** The aim of this study was to investigate the synergistic effect of a recently discovered strain *Lactobacillus casei* Qian (LC-Qian) and geniposide on the prevention of ethanol-induced gastric injury in mice. LC-Qian ( $0.5 \times 10^8$  CFU/kg  $m_b$ ) was mixed with geniposide (50 mg/kg  $m_b$ ), and LC-Qian ( $0.5 \times 10^8$  CFU/kg  $m_b$ ) and geniposide (50 mg/kg  $m_b$ ) were also used separately to protect against HCl/ethanol induced gastric injury and to compare their preventive effects. LC-Qian combined with geniposide (the combination group) diminished the gastric lesion area and gastric secretion volume and raised the pH of the gastric juice to a level close of that of the control group and significantly different from that in the model group. The combination treatment was also able to decrease the serum levels of cytokines such as interleukin-6 (IL-6), IL-12, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ), motilin (MOT), substance P (SP), and endothelin-1 (ET-1) but increase the levels of somatostatin (SS) and vasoactive intestinal peptide (VIP) as compared to the model group. The combination group showed higher NO contents in serum, heart, liver, kidney and stomach in comparison to the model group, but their levels were lower than those in the normal group. As indicated by reverse transcription polymerase chain reaction (RT-PCR) assay, the combination treatment could increase the mRNA expression levels of neuronal nitric oxide synthase (*nNOS*), endothelial NOS (*eNOS*) and inhibitor kappa B- $\alpha$  (*I $\kappa$ B- $\alpha$* ), and decrease the mRNA expression levels of inducible nitric oxide synthase NOS (*iNOS*) and nuclear factor kappa B (*NF- $\kappa$ B*) as compared to the model group. These expression levels in the combination group were similar to those of the normal group. From these results, it can be concluded that the combination treatment has the best preventive effects against gastric injury and LC-Qian could enhance the preventive effect of geniposide against gastric injury. Therefore, this combination treatment could be used as a new way method for the prevention of gastric injury.

**Key words:** *Lactobacillus casei* Qian; ethanol; mRNA expression; mouse; stomach

## 干酪乳杆菌Qian增强京尼平苷对盐酸/乙醇诱导小鼠胃损伤的预防效果

蹇宇<sup>1</sup>, 索化夷<sup>2</sup>, 易若琨<sup>1</sup>, 李贵节<sup>1</sup>, 赵欣<sup>1,\*</sup>

(1. 重庆第二师范学院生物与化学工程学院, 重庆市功能性食品协同创新中心, 重庆市功能性食品工程技术研究中心, 功能性食品研发重庆市工程实验室, 重庆 400067; 2. 西南大学食品科学学院, 重庆 400715)

**摘要:** 为探讨最近新发现的干酪乳杆菌Qian (*Lactobacillus casei* Qian, LC-Qian) 和京尼平苷组合对胃损伤预防效果的增强作用, 比较分析LC-Qian ( $0.5 \times 10^8$  CFU/kg, 以体质量计, 下同)、京尼平苷 (50 mg/kg) 以及LC-Qian ( $0.5 \times 10^8$  CFU/kg) + 京尼平苷 (50 mg/kg) 对盐酸/乙醇诱导胃损伤的预防效果。LC-Qian + 京尼平苷 (组合组) 能减少胃损伤面积和胃液量, 同时提高胃液pH值, 能使这些指标接近正常组并且与模型组显著不同。

收稿日期: 2017-04-19

基金项目: 重庆高校创新团队建设计划项目 (CXTDX201601040);

重庆市工程技术研究中心建设项目 (cstc2015yfpt\_gcjsyzjz0027);

重庆市基础与前沿研究计划一般项目 (cstc2016jcyjA0339); 重庆第二师范学院校级科研项目 (KY2015TBZC)

作者简介: 蹇宇 (1976—), 女, 副教授, 博士, 研究方向为食品化学与营养学。E-mail: qianyubaby@126.com

\*通信作者: 赵欣 (1981—), 男, 教授, 博士, 研究方向为食品化学与营养学。E-mail: zhaoxin@cque.edu.cn

相对于模型组,组合组能降低细胞因子白细胞介素-6(interleukin-6, IL-6)、IL-12、肿瘤坏死因子 $\alpha$ 和 $\gamma$ -干扰素的水平,同时降低血清胃动素、P物质、内皮素-1的水平和提高生长激素抑制素、血管活性肠肽的水平。相比模型组,在心脏、肝脏、肾脏和胃组织中组合组表现出更高的NO含量,但是这些含量低于正常组。通过逆转录聚合酶链式反应分析观察到:相对于对照组小鼠,组合组能够上调神经元型一氧化氮合酶、内皮型一氧化氮合酶、核因子 $\kappa$ B抑制蛋白 $\alpha$ 的mRNA表达和下调诱导型一氧化氮合成酶、核因子 $\kappa$ B表达,同时以上这些表达强度与正常组较为接近。通过这些结果可以看到LC-Qian和京尼平苷联合使用具有最好的胃损伤预防效果,LC-Qian能够提高京尼平苷的胃损伤预防效果,该组合可作为一种新的参考方法用于预防胃损伤。

**关键词:**干酪乳杆菌Qian;乙醇;mRNA表达;小鼠;胃

DOI:10.7506/spkx1002-6630-201721037

中图分类号:TS272.5

文献标志码:A

文章编号:1002-6630(2017)21-0230-11

引文格式:

QIAN Yu, SUO Huayi, YI Ruokun, et al. *Lactobacillus casei* Qian enhances the preventive effect of geniposide on HCl/ethanol induced gastric injury in mice[J]. 食品科学, 2017, 38(21): 230-240. DOI:10.7506/spkx1002-6630-201721037. <http://www.spkx.net.cn>

QIAN Yu, SUO Huayi, YI Ruokun, et al. *Lactobacillus casei* Qian enhances the preventive effect of geniposide on HCL/ethanol induced gastric injury in mice[J]. Food Science, 2017, 38(21): 230-240. (in English with Chinese abstract) DOI:10.7506/spkx1002-6630-201721037. <http://www.spkx.net.cn>

*Gardenia jasminoides* is a member of Rubiaceae. Its fruit is used as a traditional Chinese medicine or functional food for the functions, including liver protection, cholagogue, mitigation, hemostatic, and detumescence etc.<sup>[1-2]</sup>. *G. jasminoides* is often used as the treatment for jaundice hepatitis, sprains and contusions, hypertension, and diabetes etc.. The geniposide is a kind of iridoid glucoside, which is soluble in water and used as a main medicinal composition of *G. jasminoides*. Its content is from 3% to 8% depending on the origin<sup>[3]</sup>.  $\beta$ -Glucosaccharase can break the glycosidic bond of geniposide, which can be hydrolyzed into genipin<sup>[4]</sup>. It has been shown that geniposide has significant curative effect on diseases of digestive system, cardiovascular system and central nervous system. In addition, geniposide also has certain anti-inflammatory effects. Moreover, it can also be used to treat soft tissue injury<sup>[5]</sup>. *Lactobacillus casei* can produce large amounts of  $\beta$ -glycosidase enzymes, which are helpful to hydrolyze geniposide into genipin<sup>[6-7]</sup>. The *Lactobacillus casei* Qian (LC-Qian) detached from yak yogurt by our research team, is a kind of *Lactobacillus casei* which has significant physiological activity<sup>[8]</sup>. The combination of geniposide and LC-Qian may be beneficial to the formation of genipin.

When inflammatory diseases happen, inducible nitrite oxide synthase (iNOS) increases the synthesis of NO that ultimately leads to cell damage<sup>[9]</sup>. It was found that genipin exhibited obvious inhibitory effect on hepatic tissue

inflammation in a concentration-dependence manner<sup>[10]</sup>. The genipin could suppress NO synthesis caused by lipopolysaccharide and interferon- $\gamma$  (INF- $\gamma$ ) by inhibiting iNOS<sup>[11]</sup>. In addition, the significant anti-inflammatory effect of genipin is also seen in mice with ear swelling, foot swelling, and rheumatoid arthritis. These effects are related to genipin's inhibition on activities of inflammatory cytokines on affected regions of inflammation and reduction of content of inflammatory mediators. To be specific, controlling the NO content in body is an important mechanism for genipin to inhibit inflammation<sup>[12]</sup>.

Ethanol is a colorless organic solvent in liquid state with a special odor, which is the main ingredient of wine and alcohol drinks. High ethanol concentration can directly corrode gastric mucosa tissues and cause acute inflammation of gastric mucosa, showing the appearance of hyperemia, followed by edema, bleeding, erosion and ulcer formation. Ethanol can destroy mucous layer and mucous neck cells on surface of gastric mucosa tissues as well as the physiological environment required for normal metabolism of gastric mucosa<sup>[13]</sup>. After ethanol is metabolized, it decomposes into acetaldehyde in gastric mucosa, which then combines with gastric mucosa protein and participates in damaging gastric mucosa. Ethanol at high concentration has a quite strong dehydrated effect which congeals tissue proteins. Except for direct damage effects mentioned above, ethanol can also induce gastric mucosal lesion by enhancing factors of gastric

mucosal lesion, and on the contrary, weaken the protective factors of gastric mucosa as well as making intracellular calcium overloads<sup>[14]</sup>.

This study aimed to examine the inhibitory effect of geniposide and the combination of geniposide and LC-Qian on alcoholic gastric mucosal lesion of mice, and to understand the mechanism of prevention on alcoholic gastric mucosal lesion by LC-Qian strengthening geniposide. The results of this study will pave a theoretical foundation for further developing *G. jasminoides* as resources of medicine and food homology.

## 1 Materials and Methods

### 1.1 Experiment animal, materials and reagents

Fifty male ICR mice (7 weeks old) were purchased from the Experimental Animal Center of Chongqing Medical University (certificate number: SYXK (Yu) 2012-0001).

LC-Qian was separated from yak yoghurt from Hongyuan county (Ngawa prefecture, Sichuan province, China) by our research team. The microorganism strain was preserved at the China Center for Type Culture Collection (M2013514, Wuhan, Hubei, China).

Geniposide Shanghai Jinsui Biotechnology Co. Ltd. (China); geniposide standard Sigma-Aldrich Co. LLC. (USA); interleukin-6 (IL-6), IL-12, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ) serum cytokine kits BioLegend Inc. (USA); motilin (MOT), somatostatin (SS), substance P (SP), vasoactive intestinal peptide (VIP), endothelin (ET)-1, nitric oxide (NO) kit Beijing Puer Weiye Biotechnology Co. Ltd. (China); Trizol reagent Gene Copoeia Inc. (USA); OligodT18, RNase, dNTP, murine leukemia virus (MLV), polymerase chain reaction (PCR) primer Invitrogen (USA).

### 1.2 Instruments and equipments

D550 digital camera Canon (Japan); SevenEasy pH meter Mettler-Toledo (Switzerland); iMark microplate reader, T100 thermal cycler Bio-Rad (USA); SAS v9.1 statistical software package SAS Institute Inc. (USA).

### 1.3 Methods

#### 1.3.1 Endurance effect of lactic acid bacteria to artificial gastric juice

Artificial gastric juice was made of 0.2 g/100 mL NaCl and 0.35 g/100 mL pepsin, and the pH was adjusted to 3.0, then LC-Qian was removed by vacuum filtration. LC-Qian (5 mL) was reactivated and centrifuged at 3 000 r/min

for 10 min. The LC-Qian was collected and re-suspended in 5 mL sterile saline, then 1 mL suspension was mixed with 9 mL artificial gastric juice and incubated in a thermostatic oscillator at 37 °C. Subsequently, 200  $\mu$ L LC-Qian ( $2.0 \times 10^8$  CFU/mL) was pipetted at 0 and 3 h, poured onto a plate with MRS agar and then incubated at 37 °C for 48 h. The quantity of LC-Qian was counted and the survival rate was determined<sup>[15]</sup>.

#### 1.3.2 Determination of LC-Qian tolerance to bile salt

Reactivated LC-Qian was inoculated at MRS-thio (MRS + 0.2 g/100 mL sodium thioglycolate) broth which contained 0.0 (control), 0.3, 0.5 and 1.0 g/100 mL ox gall, respectively. Then the inoculated broth was cultured 37 °C for 24 h, the OD value of each culture was measured at 620 nm, the tolerance of the bacterial strain to ox gall was determined by comparing the OD of the ox gall tube with that of the control tube<sup>[15]</sup>.

#### 1.3.3 Determination of the hydrophobic properties of LC-Qian

Reactivated LC-Qian (5 mL,  $2.0 \times 10^8$  CFU/mL) was centrifuged at 3 000 r/min for 10 min. The LC-Qian was collected, resuspended in 5 mL phosphate buffered solution (PBS) (50 mmol/L, pH 6.5) and centrifuged at 3 000 r/min for 10 min. PBS was used as blank for absorption, the final bacterial suspension was adjusted using PBS to produce a 1.00 absorbance at 560 nm. Adjusted bacteria suspension (4 mL) was added to 0.8 mL dimethylbenzene, vibrated for 30 s and allowed to settle into layers. The aqueous layer was measured for absorbance at 560 nm<sup>[15]</sup>.

#### 1.3.4 Experiments with mice

The 50 mice were randomly divided into 5 groups with 10 mice in each group as follows: normal group, model group, LC-Qian group, geniposide group and LC-Qian + geniposide (combination) group. The experimental period lasted 2 weeks and the experiment samples were treated by gavage once a day. The mice in normal group were only treated with 0.2 mL distilled water; the mice in model group were also only treated with 0.2 mL distilled water; the mice in LC-Qian group were treated with LC-Qian ( $0.5 \times 10^8$  CFU/kg  $m_b$  (the same below)); the mice in geniposide group were treated with 50 mg/kg geniposide; combination group mice were treated with the mixture liquid of LC-Qian ( $0.5 \times 10^8$  CFU/kg) and geniposide (50 mg/kg). After being treated 14 days, all the mice were starved for 24 h from the 14<sup>th</sup> day, then the mice except normal group mice were treated by HCl/ethanol (0.1 mL/10 g  $m_b$ , 60% (V/V) in 150 mmol/L HCl) for inducing gastric injury<sup>[16]</sup>. After being treated with HCl/ethanol for 30 min, all mice were sacrificed using CO<sub>2</sub>, blood and the gastric tissues were simultaneously collected.

### 1.3.5 Mice gastric injury evaluation

The gastric secretion volume and pH of gastric juice of mice were measured by using a 10 mL measuring cylinder and pH meter respectively. The isolated stomachs were inflated by injecting 10 mL of 1% (*V/V*) formalin solution for 10 min, and the injury area of stomach was determined by digital camera and analyzed by ImageJ 1.44 software.

$$\text{Inhibitory rate}/\% = (1 - \frac{S_1}{S_2}) \times 100$$

Where  $S_1$  was gastric injury area of sample treated mice/cm<sup>2</sup>;  $S_2$  was gastric injury area of model mice/cm<sup>2</sup>.

### 1.3.6 Index determination

Serum levels of IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  were determined by enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol, and the levels of MOT, SP, SS, VIP and ET-1 were determined by radioimmunoassay kits according to the manufacturer's protocols. The NO contents in serum, heart, liver, kidney and stomach were determined by experiment kits according to the manufacturer's protocols.

### 1.3.7 Reverse transcription polymerase chain reaction assay

**Table 1 Sequences of reverse transcription-polymerase chain reaction primers used in this study**

Gene	Sequence (5'-3')
<i>nNOS</i>	Forward: GAA TAC CAG CCT GAT CCA TGG AA
	Reverse: TCC TCC AGG AGG GTG TCC ACC GCA TG
<i>eNOS</i>	Forward: GGA GAG GCT GCA TGA CAT TG
	Reverse: GGT AGA GCC ATA GTG GAA TGA C
<i>iNOS</i>	Forward: AGA GAG ATC GGG TTC ACA
	Reverse: CAC AGA ACT GAG GGT ACA
<i>NF-<math>\kappa</math>B</i>	Forward: CAC TTA TGG ACA ACT ATG AGG TCT CTG G
	Reverse: CTG TCT TGT GGA CAA CGC AGT GGA ATT TTA GG
<i>I<math>\kappa</math>B-<math>\alpha</math></i>	Forward: GCT GAA GAA GGA GCG GCT ACT
	Reverse: TCG TAC TCC TCG TCT TTC ATG GA
<i>GAPDH</i>	Forward: CGG AGT CAA CGG ATT TGG TC
	Reverse: AGC CTT CTC CAT GGT CGT GA

Total RNA was extracted from gastric tissues of mice with RNazol reagent. The extracted gastric tissue RNA was diluted to 1  $\mu$ g/ $\mu$ L. Then 1  $\mu$ L of OligodT18, RNase, dNTP, MLV enzymes and 5  $\times$  buffer (10  $\mu$ L) were added into the gastric tissue for RNA extract (2  $\mu$ L) to synthesize cDNA under three different conditions as 37  $^{\circ}$ C for 120 min, 99  $^{\circ}$ C for 4 min, 4  $^{\circ}$ C for 3 min. Then the mRNA expression of *nNOS*, *eNOS*, *iNOS*, *NF- $\kappa$ B*, *I $\kappa$ B- $\alpha$*  and *GAPDH* (Table 1) genes were amplified by the method of reverse transcription-polymerase chain reaction (RT-PCR). PCR in an automatic thermocycler was programmed as follows: 30 cycles of 94  $^{\circ}$ C for 30 s, 55  $^{\circ}$ C for 30 s, and 72  $^{\circ}$ C for 40 s, followed

extension at 75  $^{\circ}$ C for 8 min. 1% agarose gel with ethidium bromide was used for electrophoresis to check the PCR amplified products<sup>[16]</sup>.

### 1.3.8 Liquid chromatogram experiment

The geniposide and LC-Qian were mixed in lactic acid bacteria culture and the concentrations were adjusted to 2 mg/mL for geniposide, 10<sup>8</sup> CFU/mL for LC-Qian. Then the mixture was incubated at 37  $^{\circ}$ C for 24 h. Thereafter, the mixture was filtered for preparation of geniposide and LC-Qian mixture.

The geniposide and genipin standard (2 mg/mL) were prepared in 20 mL volumetric flasks, respectively. The liquid chromatogram experiment was performed under following chromatographic conditions: C<sub>18</sub> chromatographic column (2.1 mm  $\times$  50 mm, 1.7  $\mu$ m); the column temperature: 25  $^{\circ}$ C; the mobile phase: 35%–65% methanol-water; the flow rate: 1 mL/min; the detection wavelength: 238 nm; the sample volume: 2  $\mu$ L (2 mg/mL geniposide solution, geniposide and LC-Qian mixture).

### 1.4 Statistical analysis

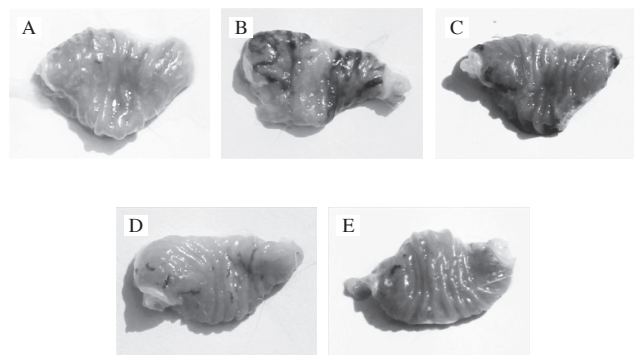
The experiments data were presented as  $\bar{x} \pm s$ . Differences in the mean values between groups were assessed with by a one-way ANOVA with Duncan's multiple range tests.  $P < 0.05$  was considered as statistically significant. The SAS v9.1 statistical software package was used for the analysis.

## 2 Results and Analyses

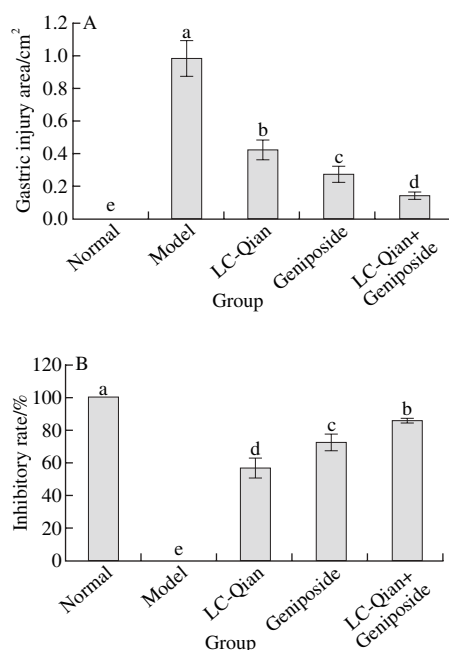
### 2.1 Biological barrier resistance and hydrophobic properties of lactic acid bacteria

LC-Qian showed the good biological barrier resistance and hydrophobic properties, the survival in artificial gastric juice of pH 3.0 was 72.33%, the hydrophobic property was 59.78% and 0.3% growth in bile salt was 22.38% (0.5% growth in bile salt was 18.72%, 1.0% growth in bile salt was 13.10%). There was a lactic acid bacteria which showed the similar biological barrier resistance and hydrophobic properties to LC-Qian, this lactic acid bacteria had the certain abilities at  $0.5 \times 10^9$  CFU/kg in mice<sup>[15]</sup>. The study showed that geniposide had the certain biological activity at concentration of 50 mg/kg in mice<sup>[16]</sup>, and another study also showed that genipin had the strong biological activity at concentration of 50 mg/kg in mice<sup>[17]</sup>, based on these results, the  $0.5 \times 10^8$  CFU/kg LC-Qian and 50 mg/kg geniposide were chosen for this study.

## 2.2 Stomach appearance of mice

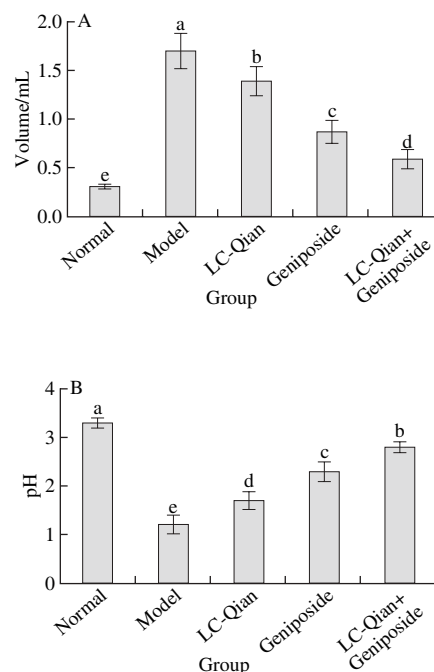


A-E. normal, model, LC-Qian, geniposide, LC-Qian + geniposide.

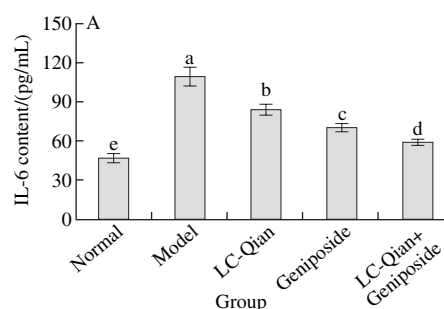
**Fig. 1** Stomachs of mice with HCl/ethanol-induced gastric injuryA. gastric injury area; B. inhibitory rate; different letters indicated significantly different ( $P < 0.05$ ) according to Duncan's multiple-range test. The same below.**Fig. 2** Inhibitory effect of LC-Qian and/or geniposide on HCl/ethanol-induced gastric injury in mice

After inducing gastric injury by HCl/ethanol solution, gastric mucosal tissues were injured; the mice in model group had the largest area of gastric injury area ( $0.98 \pm 0.11$  cm<sup>2</sup>) (Fig. 1B and Fig. 2A). LC-Qian (gastric injury area of  $0.42 \pm 0.06$  cm<sup>2</sup>, inhibitory rate of  $57.1 \pm 5.8\%$ ) and geniposide (gastric injury area of  $0.27 \pm 0.05$  cm<sup>2</sup>, inhibitory rate of  $72.4 \pm 5.2\%$ ) significantly reduced the injury area ( $P < 0.05$ ), half concentration of LC-Qian and geniposide mixture enhanced inhibitory effect on injury (gastric injury area of  $0.14 \pm 0.02$  cm<sup>2</sup>, inhibitory rate of  $85.7 \pm 1.4\%$ ), the mice treated with the mixture showed very small injury area (Fig. 2).

## 2.3 Gastric secretion volume and pH of gastric juice

**Fig. 3** Gastric secretion volume (A) and pH (B) of gastric juice in HCl/ethanol-induced gastric injury mice

The mice in model group showed the highest gastric secretion volume of  $1.70 \pm 0.18$  mL and the lowest pH of the gastric juice of  $1.2 \pm 0.2$ . On the contrary, mice in normal group ( $0.31 \pm 0.02$  mL gastric secretion volume, pH  $3.3 \pm 0.1$ ) showed the opposite situations (Fig. 3). After being treated with LC-Qian ( $1.39 \pm 0.15$  mL gastric secretion volume, pH  $1.7 \pm 0.2$ ) and geniposide ( $0.87 \pm 0.11$  mL gastric secretion volume, pH  $2.3 \pm 0.2$ ), the gastric secretion volume and pH were significantly different with the model group, the mixture of LC-Qian and geniposide significantly intensified these changes ( $P < 0.05$ ).

2.4 Levels of cytokine IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  levels in mice



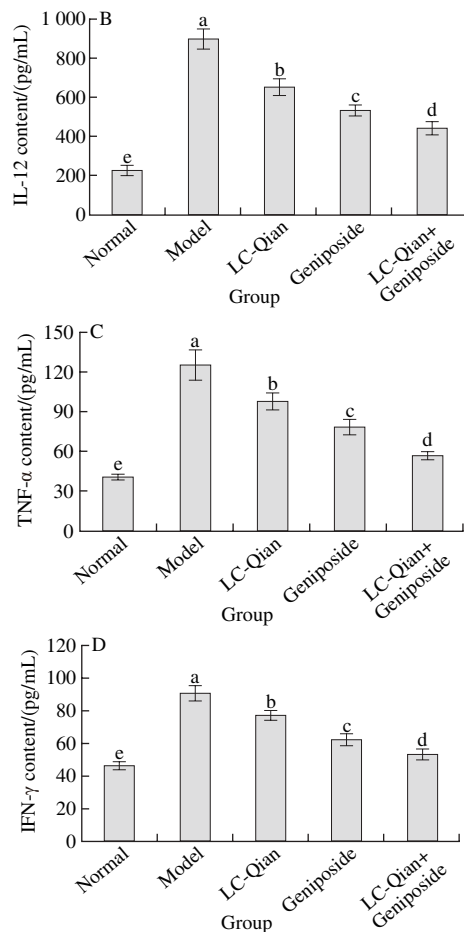


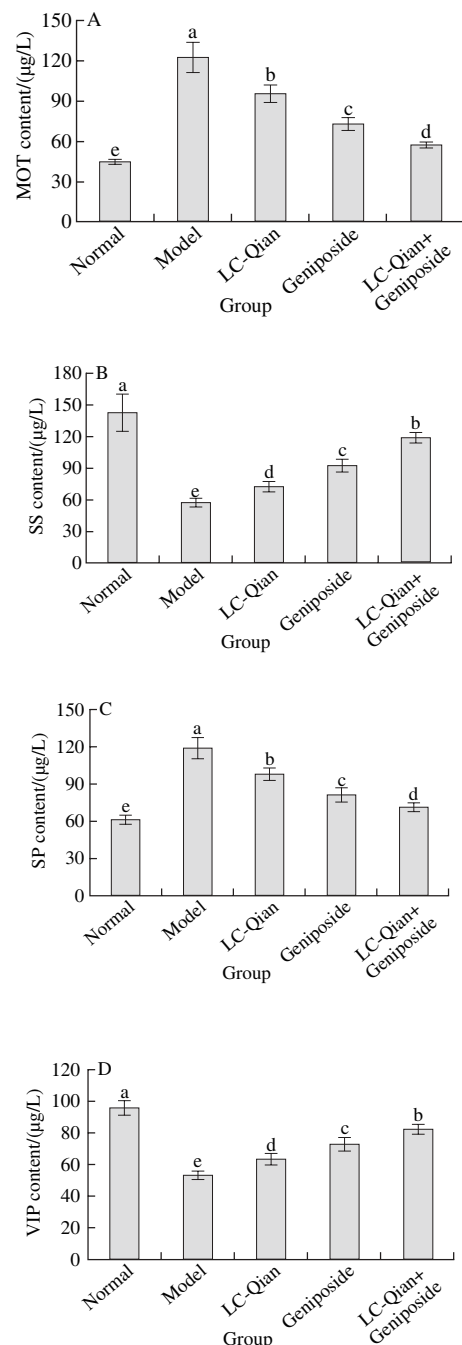
Fig. 4 Serum levels of cytokines IL-6 (A), IL-12 (B), TNF- $\alpha$  (C) and IFN- $\gamma$  (D) in mice with HCl/ethanol-induced gastric injury

The serum levels of IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  cytokine in mice of normal group ( $(47.3 \pm 3.2)$ ,  $(266.1 \pm 25.8)$ ,  $(41.1 \pm 1.8)$  and  $(46.3 \pm 2.5)$  pg/mL) were lowest as compared to those of mice in all the other groups, where these levels in mice of model group ( $(109.6 \pm 6.6)$ ,  $(898.2 \pm 50.1)$ ,  $(125.1 \pm 11.6)$  and  $(90.8 \pm 4.6)$  pg/mL) were highest (Fig. 4). Mice treated with LC-Qian and geniposide mixture ( $(59.2 \pm 2.2)$ ,  $(442.6 \pm 31.1)$ ,  $(56.9 \pm 3.0)$  and  $(53.2 \pm 3.3)$  pg/mL) had lower levels of IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  levels than those in mice treated with LC-Qian ( $(84.2 \pm 4.4)$ ,  $(651.0 \pm 42.0)$ ,  $(98.1 \pm 6.2)$  and  $(77.1 \pm 3.2)$  pg/mL) or geniposide ( $(70.5 \pm 3.1)$ ,  $(531.2 \pm 28.1)$ ,  $(78.6 \pm 5.5)$  and  $(62.3 \pm 3.5)$  pg/mL).

## 2.5 Serum levels of MOT, SS, SP, VIP and ET-1 levels in mice

The serum levels of MOT, SP, and ET-1 in mice of normal group ( $(44.5 \pm 1.9)$ ,  $(60.6 \pm 4.1)$   $\mu$ g/L and  $(68.7 \pm 2.5)$  pg/mL) were the lowest whereas the levels of SS ( $(142.1 \pm 17.2)$   $\mu$ g/L) and VIP ( $(95.8 \pm 4.7)$   $\mu$ g/L) were the highest (Fig. 5). HCl/ethanol solution (model group) could significantly change levels of MOT, SS, SP, VIP and ET-1

( $(122.0 \pm 11.2)$ ,  $(55.9 \pm 4.8)$ ,  $(118.9 \pm 7.8)$ ,  $(52.9 \pm 2.6)$   $\mu$ g/L and  $(99.1 \pm 5.3)$  pg/mL) ( $P < 0.05$ ). On the contrary, LC-Qian and geniposide could inhibit these changes, and the inhibitory effects of geniposide ( $(72.6 \pm 4.8)$ ,  $(92.1 \pm 6.3)$ ,  $(81.3 \pm 5.6)$ ,  $(72.9 \pm 4.3)$   $\mu$ g/L and  $(82.6 \pm 2.2)$  pg/mL) were stronger than those of LC-Qian ( $(95.2 \pm 6.2)$ ,  $(71.6 \pm 5.1)$ ,  $(97.6 \pm 5.2)$ ,  $(63.6 \pm 3.2)$   $\mu$ g/L and  $(88.1 \pm 2.1)$  pg/mL). After mixing half concentration of LC-Qian and geniposide, the new mixture kept MOT, SS, SP, VIP and ET-1 levels ( $(57.1 \pm 2.2)$ ,  $(118.6 \pm 4.5)$ ,  $(71.3 \pm 3.5)$ ,  $(82.6 \pm 2.9)$   $\mu$ g/L and  $(75.1 \pm 1.6)$  pg/mL) closest to those of mice in normal levels.



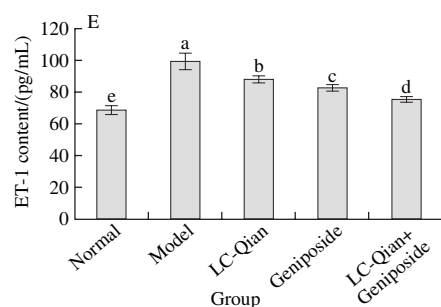


Fig. 5 Serum levels of MOT (A), SS (B), SP (C), VIP (D) and ET-1 (E) in mice with HCl/ethanol-induced gastric mucosal injury

## 2.6 NO levels in mice

The NO contents in serum, heart, liver, kidney and stomach of normal mice ( $(74.9 \pm 5.1)$ ,  $(9.2 \pm 0.5)$ ,  $(3.2 \pm 0.3)$ ,  $(9.0 \pm 0.4)$  and  $(10.7 \pm 0.6)$   $\mu\text{mol/g pro}$ ) were highest among mice in all groups (Fig. 6). Mice treated with LC-Qian and geniposide mixture ( $(64.1 \pm 2.8)$ ,  $(6.6 \pm 0.3)$ ,  $(2.5 \pm 0.2)$ ,  $(7.4 \pm 0.4)$  and  $(8.1 \pm 0.4)$   $\mu\text{mol/g pro}$ ) had the higher NO contents, which were only slightly lower than those of the normal mice. Both LC-Qian ( $(34.1 \pm 3.1)$ ,  $(2.8 \pm 0.2)$ ,  $(1.1 \pm 0.2)$ ,  $(4.1 \pm 0.3)$  and  $(3.6 \pm 0.1)$   $\mu\text{mol/g pro}$ ) and geniposide ( $(52.7 \pm 4.2)$ ,  $(4.1 \pm 0.4)$ ,  $(1.6 \pm 0.2)$ ,  $(5.6 \pm 0.4)$  and  $(5.2 \pm 0.3)$   $\mu\text{mol/g pro}$ ) treatments caused higher NO contents in the serum, heart, liver, kidney and stomach as compared to that of mice in the model group ( $(25.7 \pm 3.4)$ ,  $(1.9 \pm 0.2)$ ,  $(0.6 \pm 0.1)$ ,  $(3.1 \pm 0.3)$  and  $(3.0 \pm 0.2)$   $\mu\text{mol/g pro}$ ), and the effects of geniposide treatment were higher than those of LC-Qian treatment.

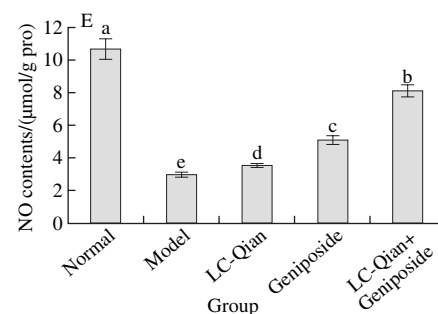
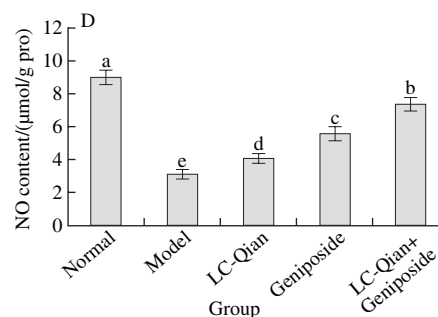
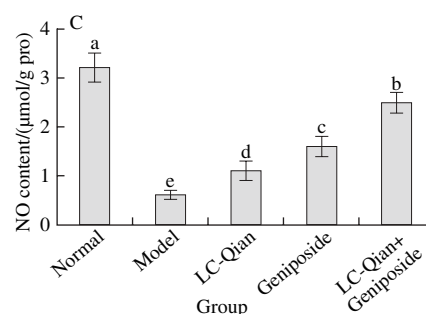
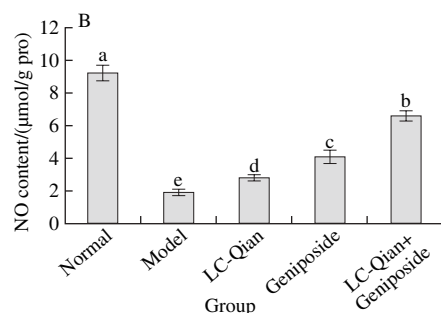
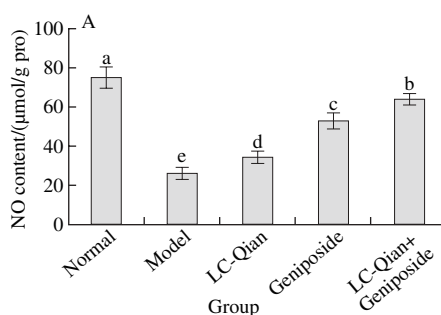
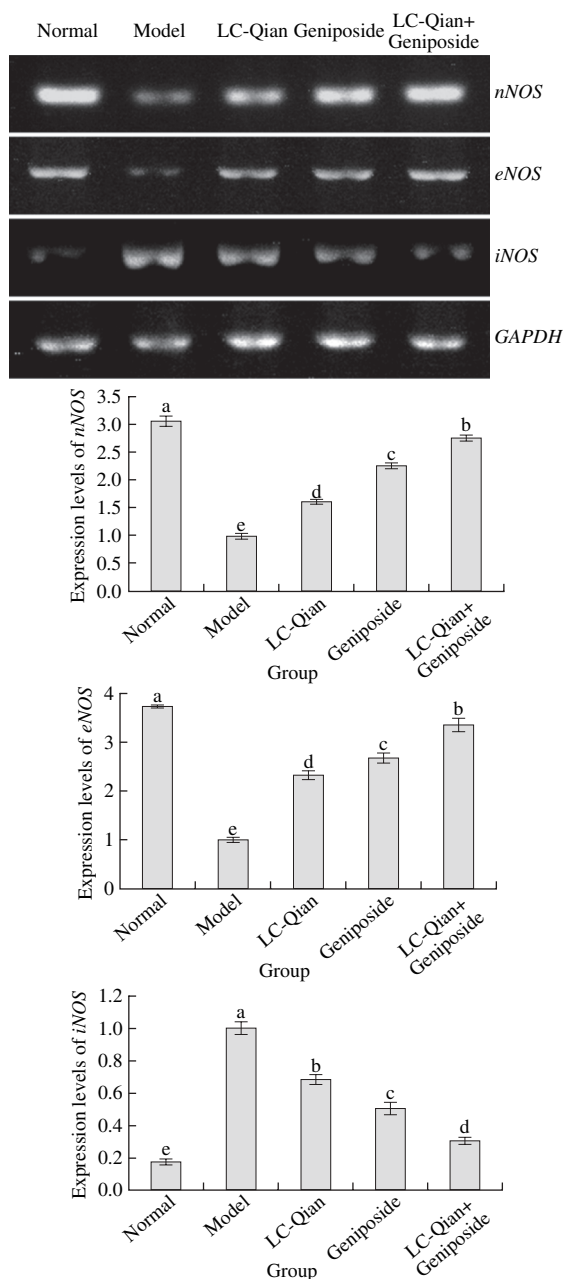


Fig. 6 NO contents in serum (A), heart (B), liver (C), kidney (D) and stomach (E) of mice with HCl/ethanol-induced gastric injury



## 2.7 The mRNA expression levels of *nNOS*, *eNOS* and *iNOS* in gastric tissue of mice

The mRNA expression of *nNOS*, *eNOS* and *iNOS* were determined by RT-PCR assay (Fig. 7), the normal mice showed the strongest *nNOS* (3.06 folds of model group), *eNOS* (3.73 folds of model group) mRNA expressions and weakest *iNOS* (0.17 folds of model group) expression. LC-Qian and geniposide treatment could increase the *nNOS*, *eNOS* expressions and decrease the *iNOS* expression as compared with the model group. Half concentration of LC-Qian and geniposide mixture could raise the effects as compared with LC-Qian or geniposide treatment, the *nNOS* (2.76 folds of model group), *eNOS* (3.35 folds of model group) and *iNOS* (0.30 folds of model group) expressions of the mixture were the closest to normal mice as compared to all other groups.



Expression level = (gene expression/GAPDH expression) × model numerical value (model fold ratio was set as 1). The same to Fig. 8.

Fig. 7 mRNA expression levels of *nNOS*, *eNOS* and *iNOS* in mice with HCl/ethanol-induced gastric injury

2.8 The mRNA expression of *NF-κB* and *IκB-α* in gastric tissue of mice

The mRNA expression of *NF-κB* of model group was highest, and expression level of *IκB-α* was lowest (Fig. 8). The mRNA expression of *NF-κB* of LC-Qian + geniposide group (0.56 folds of model group) was lower than that of LC-Qian (0.83 folds of model group) or that of geniposide group (0.81 folds of model group). The expression level of *IκB-α* in mice of these groups showed the opposite trends,

LC-Qian + geniposide group (5.36 folds of model group) had a level of *IκB-α* only lower than those of normal group (13.85 folds of model group).

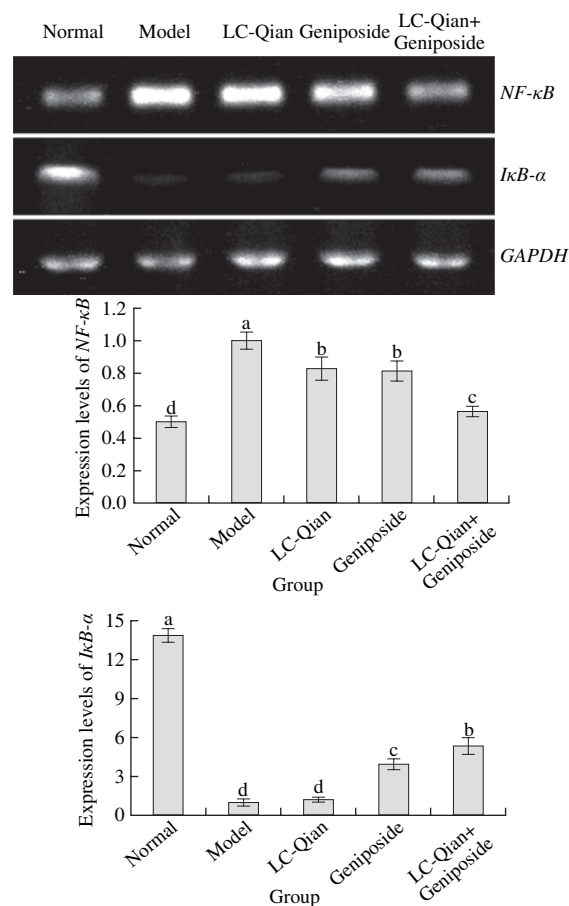


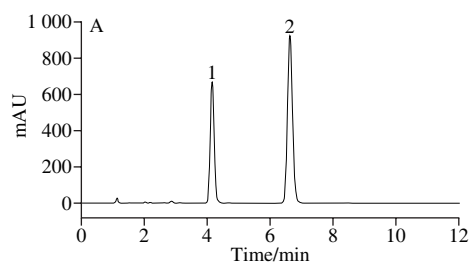
Fig. 8 mRNA expression levels of *NF-κB* and *IκB-α* in mice with HCl/ethanol-induced gastric injury

## 2.9 The activity of *in vitro* β-glycosidase enzymes

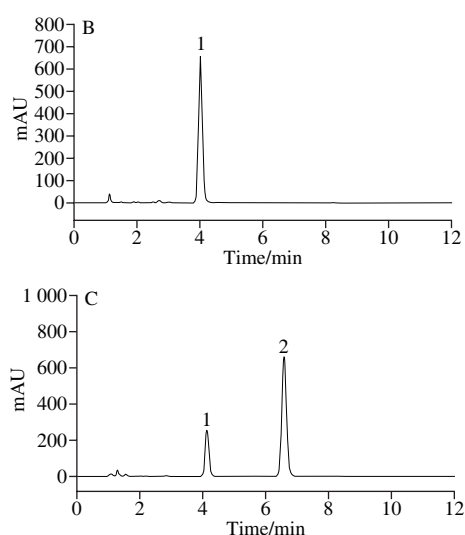
After the p-NP solution determination, the p-NP standard curve was calculated, the standard curve was  $Y = 19.571x + 0.0010$  ( $R^2 = 0.9999$ ). Control the standard curve, the β-glycosidase enzymes activity of LC-Qian was 10.21 U/mL.

## 2.10 Transformation of geniposide to genipin by LC-Qian

The geniposide could transform to genipin by β-glycosidase of LC-Qian (Fig. 9), after LC-Qian treatment for 24 h, most of geniposide (2 mg/mL, Fig. 9B) was transform to genipin (Fig. 9C).







A. chromatograms of geniposide and genipin standard;  
B. chromatograms of geniposide solution; C. chromatograms of  
LC-Qian treated geniposide solution; 1. geniposide; 2. genipin.

Fig. 9 Chromatograms of geniposide and genipin

### 3 Discussion

Ethanol can cause a great damage to gastric tissues. The damaged gastric mucosa is different from normal gastric mucosa, and the damaged sites can be intuitionistic as observed. After the gastric injury caused by ethanol, the gastric secretion volume was increased and the pH of the gastric juice was reduced<sup>[18]</sup>. In this study, the mice with gastric injury showed a large injured area. The gastric secretion volume was largest and the pH of the gastric juice was lowest. LC-Qian and geniposide could decrease the appearances of these symptoms. However, the half concentration of LC-Qian and geniposide mixture showed the significantly synergistic inhibition on ethanol-caused damage.

LC-Qian is a kind of lactic acid bacteria which was separated from yak yoghurt from Hongyuan county in China. The survival rate in pH 3.0 artificial gastric juice, hydrophobic properties and growth in bile salt of LC-Qian were higher than those of the common lactic acid bacteria (*Lactobacillus bulgaricus*). The *in vitro* properties of LC-Qian were better than those of the common lactic acid bacteria<sup>[8]</sup>. The LC-Qian also had the better *in vivo* anti-constipation effect than *Lactobacillus bulgaricus* did<sup>[8]</sup>, the *in vitro* and *in vivo* results suggest that LC-Qian may be an good quality probiotic.

IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  are all cytokines related to inflammation<sup>[19]</sup>. Gastric mucosal lesion caused by ethanol can trigger inflammation reactions. With increasing number

of lesion, inflammation becomes worse too. After tissues are damaged, endothelial cells excrete large amount of factors such as IL-6 and IL-12, which as a result, directly initiate inflammation reactions and intensifies inflammation degrees by stimulating the release of other inflammatory mediators, and then more IL-6 and IL-12 were released<sup>[20-21]</sup>. TNF- $\alpha$  is a kind of cell factor that possesses various biological activities. Plenty of it is generated and released in body and it destroys immunologic balance of organism and generates gastric mucosal lesion, which together with other inflammatory factors, becomes more harmful<sup>[22]</sup>. Cytokine IFN- $\gamma$  plays a very important role in protecting aspects of peptic ulcer and changing mucosa lesion. A large amount of IFN- $\gamma$  can aggravate gastric ulcer and reduce the protective effect on gastric mucosa caused by organism<sup>[23]</sup>. There had been researches showing that gastric mucosal lesion induced by ethanol had also led to the increased level of IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$ <sup>[18]</sup>. LC-Qian or geniposide could decrease the levels of IL-6, IL-12, TNF- $\alpha$  and IFN- $\gamma$  in mice with gastric injury, but after mixing both, their effects could be increased.

MOT and SP are excitatory gastrointestinal hormones. Their contents become high when they are stimulated. After being stimulated by ethanol, MOT and SP can cause the secretion of a large amount of gastric acid, which increases the acidity inside of stomach and intensifies the degree of gastric mucosal lesion<sup>[24]</sup>. SS and VIP are inhibitory gastrointestinal hormones, which can inhibit secretion of gastric acid. Contents of SS and VIP are decreased after tissues are damaged. They decline on inhibition of gastric acid. Gastric mucosal lesion caused by ethanol can lead to a large number of damaged tissues in gastric mucosa<sup>[25]</sup>. The amount of gastric juice is increased and pH is decreased. These iconic indicators can be taken as the keys for evaluating gastric mucosal lesion<sup>[26]</sup>. The levels of MOT, SP, SS and VIP of mice treated with LC-Qian and geniposide mixture were closest to those of normal mice.

ET is an important factor in regulating cardiovascular function. It plays crucial part in maintaining basic vascular tension and steady state of cardiovascular system. Specifically, ET-1 is mainly expressed in endothelial cells. Both ET-1 and NO are the most important vascular activity mediators generated from vascular endothelial cells. They interact and regulate blood flow of gastric mucosa<sup>[27]</sup>. NO can inhibit synthesis of excessive ET-1. ET-1 is a kind of active ingredient that causes powerful contraction of blood vessel.

There had been research testifying that ET-1 had participated in the physiopathological mechanism of gastric mucosal lesion. Due to its powerful effect of contracting blood vessels, the blood-supply quantity of gastric mucosa was declined dramatically and the protective effect of gastric mucosa was weakened<sup>[28]</sup>. LC-Qian and geniposide mixture could overcome the reduced ET-1 levels in mice with gastric injury.

NO is a kind of neurotransmitter and messenger molecule released by non-adrenergic and non-cholinergic nerves of gastrointestinal tract. It is generated by NOS catalyzer. Normally excretive NO may exert its function for protecting gastric mucosa by ways of increasing blood flow volume of gastric mucosa, maintaining the integrity of gastric mucosa epithelium, participating in protection and repairing after having mucosal lesion and inhibiting chemotaxis and adhesion of inflammatory cells<sup>[29]</sup>. The content of NO in organism is mainly regulated and controlled by NOS. There are two main categories of NOS in gastric mucosa, which are inherent NOS (cNOS, including nNOS and eNOS) that rely on  $\text{Ca}^{2+}$  and inducible NOS (iNOS) that doesn't rely on  $\text{Ca}^{2+}$ . cNOS is mainly distributed in nerve cells and endothelium cells, where it has stable activity. It helps to protect gastric mucosa by continuously releasing a small amount of NO<sup>[30]</sup>. iNOS has activity only when it is stimulated by certain cell factors (such as IL-1, IFN- $\gamma$ , and TNF- $\alpha$ ) and endotoxin. It causes NO released for a long time, aggravates inflammation and leads to a further damage to gastric mucosa<sup>[31]</sup>. LC-Qian and geniposide mixture can effectively increase expression levels of cNOS (nNOS and eNOS) in gastric tissue and reduce expression level of iNOS. This indicates that *Gardenia jasminoides* has an effect on protecting gastric mucosa.

There exists activation in NF- $\kappa$ B after gastric mucosa is damaged. This verifies that the signal transduction pathway of NF- $\kappa$ B participates in gastric mucosal lesion. NF- $\kappa$ B is an important transcription factor to mediate oxidative stress reaction. Its activation can induce expression levels of various proinflammatory cytokine and inflammatory mediators, which participate in inflammatory reaction. There often exists obvious inflammatory reaction after gastric mucosa is damaged<sup>[32]</sup>. The expression levels of TNF- $\alpha$ , COX-2 and iNOS increased obviously as well. However, their genetic transcriptions are all regulated by NF- $\kappa$ B, and this further supports that the effect of NF- $\kappa$ B signal transduction pathway when gastric mucosal lesion happens. When cells of gastric mucosa are stimulated exogenously, I $\kappa$ B phosphorylation on multimer degrades<sup>[33]</sup>. NF- $\kappa$ B is activated, and enters into cell

nucleus where it binds to the regional locus of specific gene initiating factors and start transcription of target genes such as cell factors, inflammatory factors and adhesion molecules. A large number of inflammatory cell factors are generated, which intensifies gastric mucosal lesion. In this research, by inhibiting I $\kappa$ B degradation, *Gardenia jasminoides* controlled the activation of inflammatory gene NF- $\kappa$ B<sup>[34]</sup>. And the effect of inhibiting gastric mucosal lesion can be achieved by inhibiting inflammatory cell factors. This is also a possible mechanism underlying the inhibitory effects of LC-Qian and geniposide.

*Lactobacillus casei* Qian (LC-Qian) is a new *Lactobacillus casei* isolated and identified by our research team. LC-Qian could produce  $\beta$ -glycosidase which could transform geniposide to genipin. This result was similar to that reported by Wang Yonghong et al.<sup>[6]</sup>. This research had adopted geniposide and LC-Qian combination to conduct lavage to mice with gastric mucosal lesion, and test the damaged areas of mice's gastric tissues. It had been observed that the geniposide and LC-Qian combination is able to inhibit gastric mucosal lesion remarkably. Furthermore, a molecular biological method had been used to test mice's serum and it had proved the inhibitory effect on gastric mucosal lesion caused by geniposide and LC-Qian combination. In addition, this research had also discovered that LC-Qian could greatly increase the inhibitory effect on gastric damage in body caused by geniposide. LC-Qian might convert geniposide into genipin, then more genipin exerted its effects. Therefore, LC-Qian and low concentration of geniposide combination could produce more genipin compared to the single geniposide. It had been testified that a combination of *Lactobacillus* preparations and traditional Chinese medicine could have enhanced the original physiological effect. This study had provided a new idea for further development and utilization of *Gardenia jasminoides*.

## References:

- [1] JIANG C X, CHENG J G, LUO T. Nutritional value analysis of medicinal and edible plant[J]. Journal of Anhui Agricultural Sciences, 2015, 43(11): 282-284. DOI:10.13989/j.cnki.0517-6611.2015.11.096.
- [2] MENG X L, LI H W, LI Y, et al. Advances in studies on chemical constituents and pharmacological activities of *Gardenia jasminoides*[J]. Chinese Journal of New Drugs, 2011, 20(11): 959-967.
- [3] LIU Y R, DING K Y, LIU J. Research on extraction and adsorption of geniposide from Sichuan *Gardenia*[J]. Natural Product Research and Development, 2014, 26(11): 1806; 1864-1867. DOI:10.16333/j.1001-6880.2014.11.020.

- [4] ZHENG L S, NI N, LIU X Q, et al. Study and application of geniposide and genipin[J]. Drug Evaluation Research, 2012, 35(4): 289-298.
- [5] WANG G F, WU S Y, XU W, et al. Geniposide inhibits high glucose-induced cell adhesion through the NF- $\kappa$ B signaling pathway in human umbilical vein endothelial cells[J]. Acta Pharmacologica Sinica, 2010, 31(8): 953-962. DOI:10.1038/aps.2010.83.
- [6] WANG Yonghong, SU Jian, WANG Jianfang, et al. Studies on the microbial transform ation of geniposide in gordenis[J]. Lishizhen Medicine & Materia Medica Research, 2007, 18(12): 3003-3004.
- [7] WAN Z T, YANG L J. Sereening lactic acid bacteria to produce extracellular  $\beta$ -glucosidase and the preliminary studies of the enzyme properties[J]. Food and Fermentation Industries, 2009, 35(4): 28-32. DOI:10.13995/j.cnki.11-1802/ts.2009.04.015.
- [8] ZHAO X, SUO H Y, QIAN Y, et al. Therapeutic effects of *Lactobacillus casei* Qian treatment in activated carbon induced constipated mice[J]. Molecular Medicine Reports, 2015, 12(2): 191-199. DOI:10.3892/mmr.2015.3737.
- [9] FREDERICA V, KHOSROW K, NIHARIKA N. The dual role of iNOS in cancer[J]. Redox Biology, 2015, 6: 334-343. DOI:10.1016/j.redox.2015.08.009.
- [10] MA S T, YANG D C, LI D, et al. Inhibition of uncoupling protein 2 with genipin exacerbates palmitate-induced hepatic steatosis[J]. Lipids in Health and Disease, 2012, 11(1): 154. DOI:10.1186/1476-511X-11-154.
- [11] KOO H J, SONG Y S, KIM H J, et al. Antiinflammatory effects of genipin, an active principle of gardenia[J]. European Journal of Pharmacology, 2004, 495(2/3): 201-208.
- [12] ZHOU Yanan, ZHANG Weiming, QIAN Hua, et al. Research advanceson pharmacological activities of geniposide and its derivatives[J]. Chinese Wild Plant Resources, 2011, 30(1): 1-4.
- [13] ROCCO A, COMPARE D, ANGRISANI D, et al. Alcoholic disease: liver and beyond[J]. World Journal of Gastroenterology, 2014, 20(4): 14652-14659. DOI:10.3748/wjg.v20.i40.14652.
- [14] LIEBER C S. Gastric ethanol metabolism and gastritis: interactions with other drugs, *Helicobacter pylori*, and antibiotic therapy (1957-1997): a review[J]. Alcoholism Clinical and Experimental Research, 1997, 21(8): 1360-1366.
- [15] QIAN Y, SUO H Y, DU M Y, et al. Preventive effect of *Lactobacillus fermentum* Lee on activated carbon-induced constipation in mice[J]. Experimental and Therapeutic Medicine, 2015, 61(9): 272-278. DOI:10.3892/etm.2014.2064.
- [16] ZHANG W X, CHEN Y Q, CHEN B, et al. Protective effect of geniposide on liver injury induced by triptolide[J]. Pharmacology and Clinics of Chinese Materia Medica, 2014, 30(3): 69-75. DOI:10.13412/j.cnki.zyyj.2014.03.022.
- [17] ZHANG H X. Protective effects of genipin on diabetic nephropathy in mice[J]. Herald of Medicine, 2015, 34(1): 26-30.
- [18] LU P J, HSU P I, CHEN C H, et al. Gastric juice acidity in upper gastrointestinal diseases[J]. World Journal of Gastroenterology, 2010, 16(43): 5496-5501. DOI:10.3748/wjg.v16.i43.5496.
- [19] CHEN S, ZHU K, WANG R, et al. Preventive effect of polysaccharides from the large yellow croaker swim bladder on HCl/ethanol induced gastric injury in mice[J]. Experimental and Therapeutic Medicine, 2014, 8(1): 316-322. DOI:10.3892/etm.2014.1712.
- [20] LIANG J, LI Y, LIU X L, et al. Relationship between cytokine levels and clinical classification of gastric cancer[J]. Asian Pacific Journal of Cancer Prevention, 2011, 12(7): 1803-1806.
- [21] WANG F Y, LIU J M, LUO H H, et al. Potential protective effects of *Clostridium butyricum* on experimental gastric ulcers in mice[J]. World Journal of Gastroenterology, 2015, 21(27): 8340-8351. DOI:10.3748/wjg.v21.i27.8340.
- [22] SUN L, WU Q, HAN B, et al. Mechanisms of immune injury and heterogeneity of bone marrow hematopoietic cells island in patients with auto-immuno-related hematocytopenia[J]. Journal of Immunoassay and Immunochemistry, 2014, 35(4): 378-387. DOI:10.1080/15321819.2014.899251.
- [23] LINDGREN Å, YUN C H, SJÖLING Å, et al. Impaired IFN- $\gamma$  production after stimulation with bacterial components by natural killer cells from gastric cancer patients[J]. Experimental Cell Research, 2011, 317(6): 849-858. DOI:10.1016/j.yexcr.2011.01.006.
- [24] LI G J, SUN P, WANG R, et al. Preventive effect of polysaccharide of *Larimichthys crocea* swim bladder on reserpine induced gastric ulcer in ICR mice[J]. Korean Journal of Physiology & Pharmacology, 2014, 18(2): 183-190. DOI:10.4196/kjpp.2014.18.2.183.
- [25] ZHOU Y L, WANG R, FENG X, et al. Preventive effect of insect tea against reserpine-induced gastric ulcers in mice[J]. Experimental and Therapeutic Medicine, 2014, 8(4): 1318-1324. DOI:10.3892/etm.2014.1859.
- [26] FENG Xia, ZHAO Xin. Preventive effect of *Ilex kudingcha* C.J. Tseng on gastric injury SD-rats[J]. Modern Food Science and Technology, 2014, 30(4): 21-25.
- [27] DU Y, ZHAO W, LU L, et al. Study on the antiulcer effects of *Veronicastrum axillare* on gastric ulcer in rats induced by ethanol based on tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and endothelin-1 (ET-1)[J]. Asian Pacific Journal of Tropical Biomedicine, 2013, 3(12): 925-930. DOI:10.1016/S2221-1691(13)60180-X.
- [28] LOPEZ-BELMONTE J, WHITTLE B J, MONCADA S. The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa[J]. British Journal of Pharmacology, 1993, 108(1): 73-78. DOI:10.1111/j.1476-5381.
- [29] FENG C J. Mechanism of nitric oxide synthase regulation: electron transfer and interdomain interactions[J]. Coordination Chemistry Reviews, 2012, 256(3/4): 393-411. DOI:10.1016/j.ccr.2011.10.011.
- [30] HAJREZAIE M, GOLBABAPOUR S, HASSANDARVISH P, et al. Acute toxicity and gastroprotection studies of a new schiff base derived copper (II) complex against ethanol-induced acute gastric lesions in rats[J]. PLoS ONE, 2012, 7(12): 1-11. DOI:10.1371/journal.pone.0051537.
- [31] LIU Y, GOU L S, YIN C, et al. A preliminary study on protective effect of *L*-citrulline against ischemia-reperfusion induced gastric mucosal lesions in rat[J]. Indian Journal of Pharmacology, 2012, 44(1): 31-35. DOI:10.4103/0253-7613.91863.
- [32] GUPTA S C, SUNDARAM C, REUTER S, et al. Inhibiting NF- $\kappa$ B activation by small molecules as a therapeutic strategy[J]. Biochimica et Biophysica Acta, 2010(10/11/12): 775-787. DOI:10.1016/j.bbagrm.2010.05.004.
- [33] CHOI Y H, KIM G Y, LEE H H. Anti-inflammatory effects of cordycepin in lipopolysaccharide-stimulated RAW 264.7 macrophages through Toll-like receptor 4-mediated suppression of mitogen-activated protein kinases and NF- $\kappa$ B signaling pathways[J]. Drug Design, Development and Therapy, 2014, 8: 1941-1953.
- [34] GAMBHIR S, VYAS D, HOLLIS M, et al. Nuclear factor kappa B role in inflammation associated gastrointestinal malignancies[J]. World Journal of Gastroenterology, 2015, 21(11): 3174-3183. DOI:10.3748/wjg.v21.i11.3174.