Therapeutic effect of yinchenhao decoction on cholelithiasis via mucin from gallbladder-intestine

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Abstract

Background: Cholelithiasis, known as gallstone, was a common and frequently occurring disease worldwide. Mucus was a viscoelastic protective layer lining the surface of mucosa and synthesized by specialized epithelial cells. Yinchenhao decoction was a classic prescription, first documented in *Treatise on Febrile Disease*. It had a definite and significant therapeutic effect on cholelithiasis, used as a complement to surgery. Our study aimed to investigate the pathological mechanism of cholelithiasis and the therapeutic mechanism of yinchenhao decoction via mucin from gallbladder-intestine.

Methods: The solubility of cholesterol in fasted state simulated intestinal fluid (FaSSIF) without and with mucin was tested. The experiment of supersaturation stability was designed by solvent-shift method. The animal experiment was performed by cholelithiasis model of high cholesterol diet. The stones were observed and the related lipid was tested by automatic biochemical analyzer. The mucin was detected by PCR and western blot. Statistics was analyzed using χ^2 -tests and t-tests.

Results: There was no significant difference in the solubility of cholesterol between FaSSIF without and with mucin. T_{ss} or AUC significantly increased with addition of mucin to FaSSIF ($p < 0.05$). A significant difference was observed in stone rate between the normal group and the model group ($p < 0.05$). Stone rate in the model group showed a significant difference from the yinchenhao decoction group (aspirin group) ($p < 0.05$). The level of related lipid showed a significant increase between the normal group and the model group ($p < 0.05$), while there was a significant decrease between the model group and the yinchenhao decoction group (aspirin group) ($p < 0.05$). A significant increase in the MUC5AC or MUC2 expression was observed between the normal group and the model group ($p < 0.01$). The yinchenhao decoction group (aspirin group) caused a significant decrease in the MUC5AC or MUC2 expression, compared with the model group ($p < 0.01$).

Conclusions: In cholelithiasis, the mucin in gallbladder (MUC5AC) highly expressed, shortened cholesterol supersaturation, and promoted cholesterol crystallization; the mucin in small intestine (MUC2) highly expressed, prolonged cholesterol supersaturation, and promoted cholesterol absorption. The yinchenhao decoction inhibited the expression of mucin from gallbladder-intestine for the treatment of cholelithiasis.

Background

Cholelithiasis, known as gallstone, was a common and frequently occurring disease worldwide. The treatment had always been a hot topic in medical science, such as surgery and pharmacotherapy. Gallstones were divided into cholesterol stones, pigment stones and mixed stones, according to the chemical composition. Among them, cholesterol stones accounted for the largest proportion. Cholesterol was poorly soluble in water and existed in bile in the form of bile salt-phospholipid micelles and phospholipid microcapsules. Due to the increased synthesis of endogenous cholesterol or the increased absorption of exogenous cholesterol, the cholesterol in bile concentrated to the state of supersaturation, the speed of its nucleation accelerated, and its mono-hydrate crystals aggregated to stones [1].

Mucus was a viscoelastic protective layer lining the surface of mucosa and synthesized by specialized epithelial cells. It distributed on all of the organs that were exposed to the external environment throughout the body, such as eye, respiratory, digestive, and vaginal tract et al [2, 3]. The properties of mucus (e.g., composition, structure, thickness) were different between them. Mucus contained 90–95% of water, 2–5% of glycoproteins and smaller quantities of lipids, proteins and minor amounts of electrolytes and DNA [4]. The main structural components of mucus were mucins [5]. They were a family of high molecular weight (200 kDa-200 MDa) and heavily glycosylated (up to 90%) proteins. The backbone of mucin was termed “apomucin”, decorated with a large number of O-linked oligosaccharides and a few N-glycan chains [6]. There were many subtypes such as MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC12, MUC13, MUC15, MUC16, MUC17, MUC19, MUC20. They divided into two categories: secretory mucin and membrane-bound mucin [7, 8]. Secretory mucin expressed throughout the lumen formed large polymer networks; Membrane-bound mucin on the epithelial cells was the anchor of secretory mucin networks [9].

Some studies showed that the gallbladder overexpressed MUC5AC in cholelithiasis, and mucin shortened the supersaturation and promoted the crystallization of endogenous cholesterol. Mucin in gallbladder served as the matrix for the formation and growth of stones [10–15]. On the other hand, exogenous cholesterol was absorbed through the small intestine, which mainly expressed MUC2. A recent research indicated that intestinal mucin stabilized the supersaturation and enhanced the absorption of poorly water soluble compounds [16]. But whether this effect existed in exogenous cholesterol needed to be proven. Mucin, as a potential target for the treatment of cholelithiasis, might affect the pathological process of gallstones by the two points-cholesterol crystallization and absorption.

Traditional Chinese medicine had a long history in the treatment of cholelithiasis. Yinchenhao decoction was a classic prescription, first documented in *Treatise on Febrile Disease*. It consisted of 18g *Artemisiae scopariae herba*, 12g *Gardeniae fructus* and 6g *Rhei radix et rhizome*, and had the effect of clearing heat, eliminating dampness and removing jaundice. Modern clinical researches also testified that yinchenhao decoction had a definite and significant therapeutic effect on cholelithiasis [17, 18], which improved clinical symptoms, regulated lipid levels, stimulated bile secretion, promoted the dissolution of gallstones, or slowed down the formation of gallstones. It was used as a complement to surgery [19–21]. However, it was not clear whether it had a mechanism of prevention of gallstone based on mucin regulation. Hypotheses were proposed: Mucin in gallbladder shortened the supersaturation and promoted the crystallization of endogenous cholesterol; mucin in small intestine prolonged the supersaturation and promoted the absorption of exogenous cholesterol, thereby inducing cholelithiasis. Yinchenhao decoction prevented the endogenous cholesterol crystallization and exogenous cholesterol absorption by the regulation of mucin expression from gallbladder-intestine, thereby treating cholelithiasis. By purified mucin and gallstone model experiment, the influence of mucin on the stability of cholesterol supersaturation in small intestine was investigated; the influence of yinchenhao decoction on the expression of MUC5AC and MUC2 was tested; the inhibition of yinchenhao decoction on the crystallization and the absorption of cholesterol was verified. The purpose of this subject was to investigate the pathological mechanism of cholelithiasis and the therapeutic mechanism of yinchenhao

decoction via mucin from gallbladder-intestine. It could discover the new action way of yinchenhao decoction and promote the modernization of traditional Chinese medicine.

Methods

Preparation of yinchenhao decoction

Yinchenhao decoction consisted of three herbs, and the mixed proportion was illustrated in Fig. 1A–B. The herbal names were checked in accordance with www.theplantlist.org (Fig. 1C). Herbs of *Artemisiae scopariae herba*, *Gardeniae fructus* and *Rhei radix et rhizoma* were obtained from pharmacy department in Tianjin NanKai Hospital (China). They were stored at room temperature. Botanical identity was authenticated by the executive manager of pharmacy department in Tianjin NanKai Hospital. Voucher specimens with storage code were deposited at pharmacy department in Tianjin NanKai Hospital. Herbs were boiled together in 6×volume of water for 30min, and then the residue was boiled in 8×volume of water for 25 min. The filtered solution was combined and concentrated into 4 g/mL. The extract was got.

Chemicals and drugs

Cholesterol was obtained from Sigma Co., Ltd (US). Aspirin (98% pure) was purchased from Sigma Co., Ltd (US). FaSSIF/FeSSIF/FaSSGF was purchased from Biorelevant.com (UK). Mucin Type II from porcine stomach was purchased from Sigma Co., Ltd (US). HPLC-grade methanol and acetonitrile were provided by Concord Co., Ltd (China). Pure water was prepared using water purification system. All other reagents and solvents were of analytical grade or better.

Animals

Male C57 mice (weight 20 g; age 2 months) were provided by HFK Bioscience CO., LTD (Beijing, China). The mice were maintained on 12 h light-dark cycle and fasted 12 h before experiment with free access to water. The Animal Ethical and Welfare Committee in Tianjin NanKai Hospital (Approval No. NKYY-DWLL-2020-063) approved the animal study. The animal study adhered to the principles for laboratory animal use and care (NIH publication #85 – 23, revised in 1985).

Test of solubility

Fasted state simulated intestinal fluid (FaSSIF) could simulate the small intestine environment in fasted state. Excessive cholesterol was added in FaSSIF without and with mucin and dissolved at 37 °C at 100 rpm for 2 days in shaker to saturation. The solutions were centrifuged and detected by HPLC.

Preparation of supersaturated solution by solvent-shift method

Cholesterol was pre-dissolved in methanol. A certain amount of solution was gradually delivered in FaSSIF without and with mucin at 37 °C at 100 rpm in shaker to supersaturation.

Experiment of supersaturation stability

This experiment was designed for testing the supersaturation stability and precipitation. The supersaturated solutions were placed at 37 °C at 100 rpm in shaker. At various time points, 600 µL samples were withdrawn from the supersaturated solutions and immediately centrifuged at 14000 r/min for 10 min. 300 µL supernatants were withdrawn and added in equivalent DMSO to stop subsequent precipitation. The solutions were detected by HPLC.

HPLC analysis

The concentration of cholesterol was determined by HPLC. A C18 column (4 µm, 3.9 mm × 75 mm, Waters, USA) with an C18 guard column (4 µm, 4 mm × 3.0 mm, Waters, USA) was maintained at 37°C. The analytical mobile phase consisted of water and acetonitrile by a 5:95 (v/v) ratio. The flow rate was 1 mL/min. The injection volume was 10 µL. Cholesterol was detected by absorbance at 205 nm.

Animal experiment

C57 mice were divided into 4 groups at random: normal group, cholelithiasis model group by high cholesterol diet, aspirin group, and yinchenhao decoction group. Aspirin was selected as positive drug because it inhibited the mucin secretion from mucosa for preventing the formation of cholesterol stones [22, 23]. The normal group was fed with normal pellet diet; The model group, aspirin group, and yinchenhao decoction group were fed with pellet diet containing 1.2% cholesterol. The normal group and model group were assessed to water by oral gavage; The aspirin group and yinchenhao decoction group were assessed to aspirin and yinchenhao decoction by oral gavage. They were fed for 8 weeks with free access to water and observed regularly. The death was recorded.

Rats were anaesthetized for the laparotomy and the blood was drawn from the abdominal aorta. The concentration of triglycerides, cholesterol and bile acids in blood was determined by automatic biochemical analyzer. The gallbladder ducts were ligated. The gallbladders were separated and cut. Stones were observed. The gallbladders were stored at -80°C for the extraction of gallbladder mucin. The small intestines were separated and cut. They were stored at -80°C for the extraction of small intestine mucin. The mucin was detected by PCR and western blot.

PCR analysis

Total RNA was extracted from the separated tissue by RNAsimple Total RNA kit (Tiangen, China), according to the manufacturer's instructions. The integrity of RNA samples was checked electrophoretically on agarose gels stained with ethidium bromide. TIANscript RT kit (Tiangen, China) was used for reverse transcription of total RNA. To quantify the cycle threshold (*C_t*) value, real-time PCR was performed on iQ5 Multicolor Real-time PCR Detection System (BIO-RAD, California, US). The primers for MUC5AC (forward 5'-GGCCAATGCGGCACTTGTACCAAT-3' and reverse 5'-GTCATCTGGACAGAAGCAGCCCTC-3'), MUC2 (forward 5'-TTCTTGCTGGGTGAAGAGTG-3' and reverse 5'-AGACAAGGTGGAGTCCAAGC-3'), and β-actin (forward 5'-CCCATCTATGAGGGTTACGC-3' and reverse 5'-TTTAATGTCACGCACGATTTC-3') were synthesized by Sangon Biotech (Shanghai, China). β-actin was used for normalization.

Western blot analysis

The separated tissue was homogenized using RIPA lysis buffer, and centrifuged at 12000 g for 5 min at 4°C. The resulting supernatants were carefully removed and stored at -80°C until use. The concentration of protein was determined by the BCA Assay Protein kit. For gel electrophoresis, 50 µg of total protein of each sample was added in Protein Loading Dye, and denatured for 5 min at 95°C. Samples were separated by electrophoresis in an 8% SDS-PAGE, and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% nonfat milk in TBST for 2 h at room temperature. For detection of interest protein, the blots were incubated with the primary antibodies and the secondary antibody. After washing with TBST, the membrane was detected by the enhanced Chemiluminescent HRP Substrate according to the manufacturer's protocol and exposed by ChemiDoc Imaging Systems (Bio-Rad, USA). β-actin was used as internal reference. The optical densities (*OD*) for the bands (MUC5AC, MUC2) of interests were determined using the Quantity One soft-ware (Bio-Rad, USA).

Data analysis

Supersaturation ratio (SSR_t)

$$SSR_t = \frac{C_t}{C_{max}} \times SSR$$

SSR_t was the supersaturation ratio value at that time point; C_t was the concentration at that time point; C_{max} was the max concentration; SSR was the supersaturation ratio of the experiment.

Time post-spiking (T_{ss})

T_{ss} was the length of time that the concentration remains above 90% of max concentration. It was a measure of the stability of supersaturation.

Area under curve (AUC)

AUC was the area under the curve from zero to time t and calculated by trapezoidal rule. It was also a measure of the stability of supersaturation.

Relative gene expression (RGE)

$$RGE = 2^{-(\Delta Ct_{TS} - \Delta Ct_{CS})}$$

ΔCt_{TS} was the difference between the Ct of target gene and reference gene of test sample; ΔCt_{CS} was the difference between the Ct of target gene and reference gene of control sample.

Relative protein expression (RPE)

$$RPE = OD\ TB / OD\ RB$$

OD TB was the *OD* of target band; *OD RB* was the *OD* of reference band.

Statistics

All results were expressed as Means $\pm$ SD. The difference of constituent ratio between different groups was analyzed for significance using χ^2 -tests. The difference of parameters between different groups was analyzed for significance using t-tests. Statistical significance was considered at $p < 0.05$, or 0.01 .

Results

Effect of mucin on solubility of cholesterol

Solubility of cholesterol ($n = 3$) was 3.39 ± 0.16 $\mu\text{g/mL}$ in FaSSIF; 3.59 ± 0.11 $\mu\text{g/mL}$ in FaSSIF Containing 0.2% Mucin. There was no significant difference in the solubility of cholesterol between FaSSIF without and with mucin.

Effect of mucin on supersaturation of cholesterol

In Fig. 2, cholesterol yielded a T_{ss} ($n = 3$) of 35 ± 8.66 min in FaSSIF; 100 ± 17.32 min in FaSSIF Containing 0.2% Mucin. AUC ($n = 3$) was 2017.71 ± 87.29 in FaSSIF; 2272.94 ± 53.25 in FaSSIF Containing 0.2% Mucin. A significant increase was observed in T_{ss} or AUC between FaSSIF Containing 0.2% Mucin and FaSSIF alone ($p < 0.05$).

General status of mice

During the experiment, mice in the normal group gradually gained weight, with shiny hair, sensitive reaction, agile activity, good spirit, and no abnormalities in the color and amount of urine or feces. The weight of mice in the model group increased rapidly in a short period, followed by slow growth, the hair was from shiny to sparse and lackluster, the spirit was from good to bad, the reaction was from sensitive to dull, the motor function was from good to bad, the appetite was from exuberant to decreased, and the legs were occasionally stained with yellow. No animals died. Mice in the aspirin group, the yinchenhao decoction group had bright hair close to skin, good reaction, activity and mental state, and slight weight loss.

In Fig. 3, after dissection, the liver and gallbladder in each group were observed under naked eye or stereomicroscope. In the normal group, the liver of mice had dark red and shiny color, sharp edge, and soft texture. The gallbladder was small, normal in appearance, and contained clear bile. In the model group, the liver had fat and hypertrophic appearance, light yellow or gray color, and fragile texture. The gallbladder had significantly increased volume, or obvious cholestasis with turbidity. The large or small, milky white or light yellow stones were seen through the gallbladder wall. In the aspirin group, the yinchenhao decoction group, the changes in liver and gallbladder were more complex. The status in most

of the liver and gallbladder was between the normal group and the model group. Few of them showed moderate damage and stones.

Stone status of mice

In Table 1, a significant difference was observed in stone rate between the normal group and the model group, $\chi^2 = 30.000$, $p < 0.05$. Stone rate in the model group showed a significant difference from the aspirin group, $\chi^2 = 15.000$, $p < 0.05$. There was a significant difference between the model group and the yinchenhao decoction group, $\chi^2 = 12.857$, $p < 0.05$.

Table 1
Stone in different groups (n = 15).

	Total case	Stone case	Stone rate (%)
Normal group	15	0	0
Model group	15	15	100
Aspirin group	15	5	33.3
Yinchenhao decoction group	15	6	40

Lipid level

In Table 2, the concentration of TBA, TC, or LDL-C of the model group showed a significant increase from the normal group ($p < 0.05$), while TRIG or HDL-C showed no significant difference. The concentration of TBA or TC of the aspirin group showed a significant decrease from the model group ($p < 0.05$), while TRIG, HDL-C, or LDL-C showed no significant difference. There was a significant decrease in the concentration of TBA or TC between the model group and the yinchenhao decoction group ($p < 0.05$), except for no significant difference in TRIG, HDL-C, or LDL-C.

Table 2
Lipid level in different groups (n = 15).

C (mmol/L)					
	TBA	TRIG	TC	HDL-C	LDL-C
Normal group	15.73 ± 4.81	0.78 ± 0.08	2.74 ± 0.69	1.92 ± 0.39	0.60 ± 0.21
Model group	60.39 ± 20.43a	0.70 ± 0.08	7.55 ± 0.52a	2.18 ± 0.27	4.08 ± 0.43a
Aspirin group	34.84 ± 21.14b	0.59 ± 0.07	6.13 ± 0.61b	2.01 ± 0.45	4.12 ± 0.58
Yinchenhao decoction group	35.81 ± 15.62b	0.66 ± 0.08	6.92 ± 0.64b	2.34 ± 0.28	4.49 ± 0.53
a vs Normal group, p < 0.05; b vs Model group, p < 0.05					

Mucin expression

In Fig. 4, a significant increase in the *RGE* of MUC5AC was observed between the normal group and the model group ($p < 0.01$). The aspirin group caused a significant decrease in the *RGE* of MUC5AC, compared with the model group ($p < 0.01$). The yinchenhao decoction group had 1.68-fold significantly less MUC5AC than the model group ($p < 0.01$).

In Fig. 5, a significant increase in the *RGE* of MUC2 was observed between the normal group and the model group ($p < 0.01$). The aspirin group caused a significant decrease in the *RGE* of MUC2, compared with the model group ($p < 0.01$). The yinchenhao decoction group had 1.58-fold significantly less MUC2 than the model group ($p < 0.01$).

In Fig. 6, there was a significant increase in the *RPE* of MUC5AC between the normal group and the model group ($p < 0.01$). The aspirin group caused a significant decrease in the *RPE* of MUC5AC, compared with the model group ($p < 0.01$). A significant decrease in the *RPE* of MUC5AC was observed between the model group and the yinchenhao decoction group ($p < 0.01$).

In Fig. 7, there was a significant increase in the *RPE* of MUC2 between the normal group and the model group ($p < 0.01$). The aspirin group caused a significant decrease in the *RPE* of MUC2, compared with the model group ($p < 0.01$). A significant decrease in the *RPE* of MUC2 was observed between the model group and yinchenhao decoction group ($p < 0.01$).

Discussion

Mucin, a main component of mucus, was a polymer, and widely existed in the body. It was known as a natural barrier to prevent pathogen contact with underlying tissue [24]. It also served many physiological functions: lubrication for the passage of objects, maintenance of a hydrated layer over the epithelium, as

a permeable gel layer for the exchange of gases and nutrients [3], and transduction of cell signaling [25]. Mucin physically and chemically interacted with each other or with other components of mucus [26]. As pH change, the mucin tended to aggregate and gel, and exhibited single molecules-aggregates transition [27, 28]. The salt bridges, formed between negatively charged carboxylates and positively charged amino groups of amino acid side chains, were broken when negatively charged carboxylates were protonated at low pH, leading to aggregation of mucin fibers [3, 29]. Alterations of mucin in physical state, especially in glycosylation patterns and expression levels, were thought to be associated with special physiological changes or diseases. The overproduction of mucin was involved in infection, inflammation, cystic fibrosis, bronchitis, asthma, gallstones and carcinoma. The underproduction of mucins was present in dry eye syndromes and ulcer [3]. These could be used as disease biomarkers.

This research focused on the relationship between mucin and gallstone, as well as the therapeutic mechanism of yinchenhao decoction on cholelithiasis. Literatures said that mucin in gallbladder significantly shortened the supersaturation and promoted the crystallization of cholesterol [10–15]. Also, our in vitro experiment demonstrated that mucin in small intestine significantly prolonged the supersaturation and delayed the precipitation of cholesterol, without the change of solubility. The established animal model experiment indicated that the yinchenhao decoction or aspirin had certain curative effect and showed that the general status improved, the stone rate decreased, and the lipid level decreased significantly. The mechanism was as below. In cholelithiasis, MUC5AC highly expressed and promoted cholesterol crystallization; MUC2 highly expressed and promoted cholesterol absorption. The yinchenhao decoction or aspirin significantly inhibited the expression of MUC5AC and the crystallization of cholesterol in cholelithiasis; The yinchenhao decoction or aspirin significantly inhibited the expression of MUC2 and the absorption of cholesterol in cholelithiasis. In this way, the mucin from gallbladder-intestine affected the formation of gallstones in the process of cholesterol crystallization and absorption. Yinchenhao decoction treated the cholelithiasis via the regulation of mucin from gallbladder-intestine. This research provided the target for treatment of cholelithiasis, the scientific reference for rational drug use in clinic, and the theoretical foundation for prescription promotion and drug development.

One question was that the material basis of yinchenhao decoction remained unclear. The formula contained *Artemisiae scopariae herba*, *Gardeniae fructus* and *Rhei radix et rhizome*. Main ingredients of *Artemisiae scopariae herba* included coumarins (6, 7-dimethoxy coumarin, scopoletin, 6-hydroxy-7-methoxy coumarin, capilarin), chromones (capillarisin, 7-methyl capillarisin, 4'-methyl capillarisin, 6-demethoxy-4'-methyl capillarisin), flavonoids (areapillin, isoareapillin, cirsimaritin), organic acids (chlorogenic acid), volatile oils and polysaccharides [30, 31]. Main ingredients of *Gardeniae fructus* included iridoid glycosides (geniposide), organic acids (chlorogenic acid), pigments (crocin, crocetin), volatile oils and polysaccharides [32, 33]. Main ingredients of *Rhei radix et rhizome* included anthraquinones (chrysophanol, rhein, emodin, physcion, aloe-emodin), tannins (procyanidine), and polysaccharides [34, 35].

The next step will be designed to screen the active ingredients for clarifying the material basis, based on separated compound prescription. It will be the subject of future research.

Conclusions

In cholelithiasis, the mucin in gallbladder (MUC5AC) highly expressed, shortened cholesterol supersaturation, and promoted cholesterol crystallization; the mucin in small intestine (MUC2) highly expressed, prolonged cholesterol supersaturation, and promoted cholesterol absorption. The yinchenhao decoction inhibited the expression of mucin from gallbladder-intestine for the treatment of cholelithiasis.

Abbreviations

FaSSIF: Fasted state simulated intestinal fluid; PVDF: Polyvinylidene difluoride; *SSR*: Supersaturation ratio; T_{ss} : Time post-spiking; *AUC*: Area under curve; *RGE*: Relative gene expression; *RPE*: Relative protein expression

Declarations

Acknowledgments

Not applicable.

Author contributions

WL and YC designed the study. WL, JL, TW, and DL performed the study. WL analyzed the data and wrote the manuscript. YC funded the study. All authors revised the manuscript and approved the final version.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Animal Ethical and Welfare Committee in Tianjin NanKai Hospital (Approval No. NKYY-DWLL-2020-063) approved the animal study. The animal study adhered to the principles for laboratory animal use and care (NIH publication #85-23, revised in 1985).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Figures

Figure 1

Information of components in yinchenhao decoction. A: the representative photograph of herbs; B: the ratio of each herb; C: the list of herbal names

Figure 2

SSR of cholesterol in FaSSIF as a function of mucin. Each point represents the mean $\pm$ SD (n=3).

Figure 3

Stereomicrograph of gallstone. A: Normal group; B: Model group; C: Aspirin group; D: Yinchenhao decoction group

Figure 4

RGE of MUC5AC in different groups (n=6). aa vs Normal group, $p<0.01$; bb vs Model group, $p<0.01$

Figure 5

RGE of MUC2 in different groups (n=6). aa vs Normal group, $p < 0.01$; bb vs Model group, $p < 0.01$

Figure 6

RPE of MUC5AC in different groups (n=6). A: the representative western blot analysis; B: the semiquantitative densitometric analysis (aa vs Normal group, $p<0.01$; bb vs Model group, $p<0.01$)

Figure 7

RPE of MUC2 in different groups (n=6). A: the representative western blot analysis; B: the semiquantitative densitometric analysis (aa vs Normal group, $p<0.01$; bb vs Model group, $p<0.01$)

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