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Baicalin protects against ethanol-induced chronic gastritis in rats by inhibiting Akt/NF-κB pathway

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ABSTRACT

Aims: Currently, chronic gastritis is a high incidence of digestive diseases, along with loss of appetite, abdominal pain and diarrhea. Baicalin belongs to the major bioactive flavonoids compounds from Scutellariae Radix, it exhibited anti-inflammatory and anti-bacteria activities. Nonetheless, the protective effects of baicalin on ethanol-induced gastritis have not been completely clarified. Our study was designed to evaluate the protective activity of baicalin on ethanol-induced chronic gastritis.

Main methods: Rat with chronic gastritis model was induced by the administration of 56% ethanol for four weeks. Baicalin (50 and 100 mg/kg) were orally administered for seven days to evaluate its curative effect, respectively. The production of TNF-α, interleukin (IL)-8, IL-1β, NO, ET-1, PGE2, LDH and COX-2 were determined by ELISA. The activities of Akt, p-Akt, IκBα, p-IκBα, NF-κBp65 and NF-κBp-p65 were tested by western blot. Immunofluorescence staining was employed to assess the location of NF-κBp65.

Key findings: The changes of the histopathological analysis and the levels of NO, ET-1, PGE2, LDH and COX-2 demonstrated that baicalin treatment ameliorated ethanol-induced gastritis. ELISA analysis showed that baicalin inhibited the levels of TNF-α, IL-8 and IL-1β. Besides, Akt, p-Akt, IκBα, p-IκBα, NF-κBp65 and NF-κBp-p65 expression were significantly suppressed by baicalin. Meanwhile, baicalin suppressed the translocation of NF-κBp65 to the cell nucleus through immunofluorescence staining, molecular docking analysis showed that baicalin had affinity with Akt and NF-κBp65.

Significance: All results demonstrated that baicalin effectively alleviated chronic gastritis via suppressing the levels of inflammatory regulators and inhibiting Akt/NF-κB activation.

1. Introduction

Chronic gastritis is currently a high incidence of digestive diseases, and the age of onset is gradually younger. Report indicated that Ethanol could induce the gastritis and peptic ulcer diseases [1]. Ethanol interferes with the secretion of cyclooxygenase (COX), lactate dehydrogenase (LDH), cytokines and oxygen-derived free radicals in the gastric mucosa, which directly or indirectly damages the gastric mucosal [2–4]. Studies showed that prostaglandin synthesis (PGs) also were involved in protecting the gastric mucosa [5]. Ethanol intake could induce the inflammation of the gastric epithelium by increasing the expression levels of tumor necrosis factor-α (TNF)-α, interleukin-6 (IL)-1β, interferon (IFN)-γ and IL-8, which are pro-inflammatory cytokines [6,7]. Therefore, this disease can be treated by effectively suppressing the occurrence of inflammatory response.

Scutellaria Radix, a Traditional Chinese Medicine, is a crucial herb of clinical curing gastritis, ulcerative colitis, dysentery and hepatitis [8]. Many classic traditional Chinese medicine prescriptions have been applied in treating gastroenteritis, ulcerative colitis or protecting gastric, which contain Scutellaria Radix, such as Banxia Xiexin decoction [9] and San-huang-xie-xin-tang [10]. In addition, baicalin is the major bioactive compound of Scutellaria Radix and belongs to bioactive flavonoids compound, which has been reported to perform some pharmacological activity, including anti-inflammatory, anti-ulcerative colitis and antioxidant [11]. However, there has no report about protective effects of baicalin on ethanol-induced gastritis. Since baicalin performs obvious anti-inflammatory activity on chronic inflammatory processes dependent on signaling pathways [12], baicalin might

Abbreviations: NF-κB, nuclear factor-kappaB; TNF-α, tumor necrosis factor-α; Akt, protein-serine- threonine kinase; ET-1, Endothelin-1; PGE_2, prostaglandin E2; LDH, lactate dehydrogenase; COX-2, cyclooxygenase-2; IFN-γ, interferon-γ; eNOS, endothelial NO synthase; PI3K, phosphatidylinositol-3-kinases

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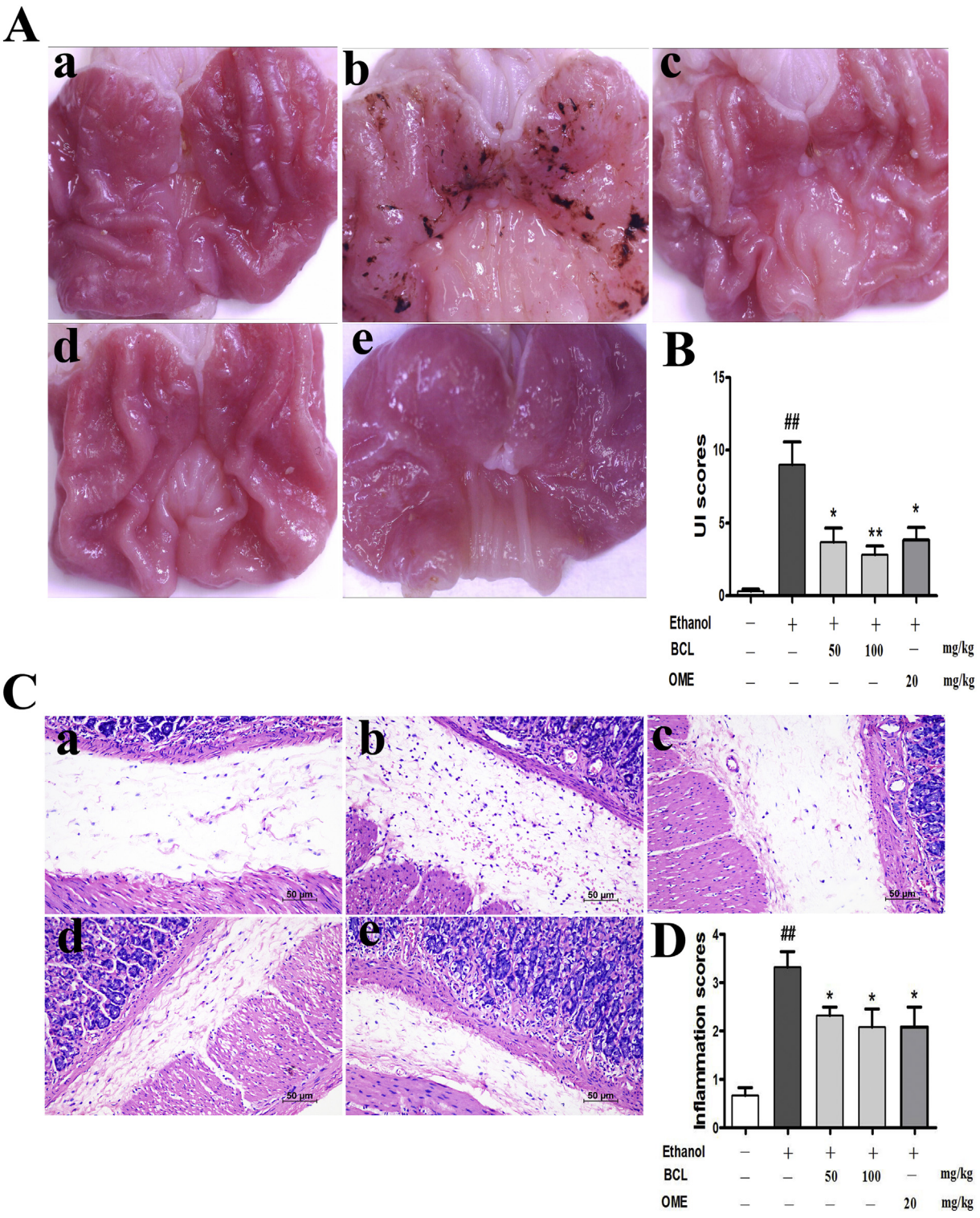

Fig. 1. Effects of baicalin on ethanol-induced chronic gastritis in SD rats. (**A**) The gross anatomy of gastric mucosa: (**a**) Normal group; (**b**) Ethanol-induced gastritis group; (**c**) BCL-treated group (50 mg/kg); (**d**) BCL-treated group (100 mg/kg); (**e**) OME group. (**B**) The gastric mucosa ulcer index (UI) evaluations during the gastritis process. (**C**) H & E staining of stomach tissues in different groups (×200 magnification): (**a**) Normal group; (**b**) Ethanol-induced gastritis group; (**c**) BCL-treated group (50 mg/kg); (**d**) BCL-treated group (100 mg/kg); (**e**) OME group. (**D**) The inflammatory scores were evaluated in the gastritis process. Data are presented as mean ± SD ($n = 6$). $^{\#\#}P < 0.01$ vs. Control group, $^{**}P < 0.01$ vs. Ethanol group, $^{*}P < 0.05$ vs. Ethanol group.

become a new drug for treating chronic gastritis. So our study was designed to assess baicalin's therapeutic effect on ethanol-induced gastritis.

Study demonstrated that activated inflammatory cells can initiate a series of intracellular signaling by inhibiting the activation of IκB kinase (IκK) and nuclear factor-kappaB (NF-κB), while increasing the expression of NO and prostaglandin E2 (PGE_2) that belongs to inflammatory factors [13]. In-depth analysis of the anti-inflammatory effects of drugs is of great significance for the treatment of chronic diseases. Previous research showed that baicalin could suppress the secretion levels of

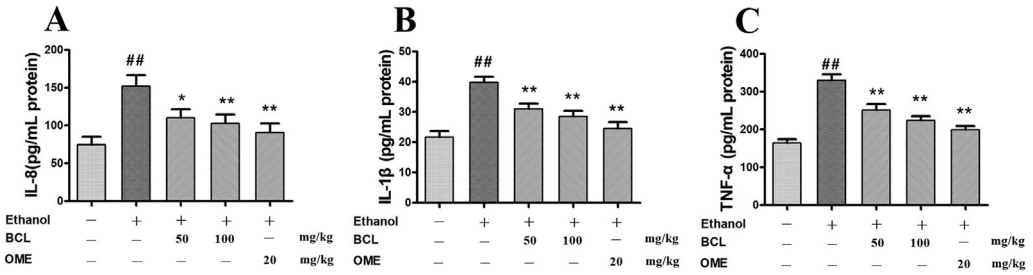

Fig. 2. Baicalin attenuated inflammatory response in ethanol-induced chronic gastritis rats. The production of inflammation-related cytokines were determined by ELISA in triplicate: (**A**) IL-8; (**B**) IL-1β; (**C**) TNF-α; data are presented as mean ± SD ($n = 6$). $^{\#\#}P < 0.01$ vs. Control group, $^{**}P < 0.01$ vs. Ethanol group, $^{*}P < 0.05$ vs. Ethanol group.

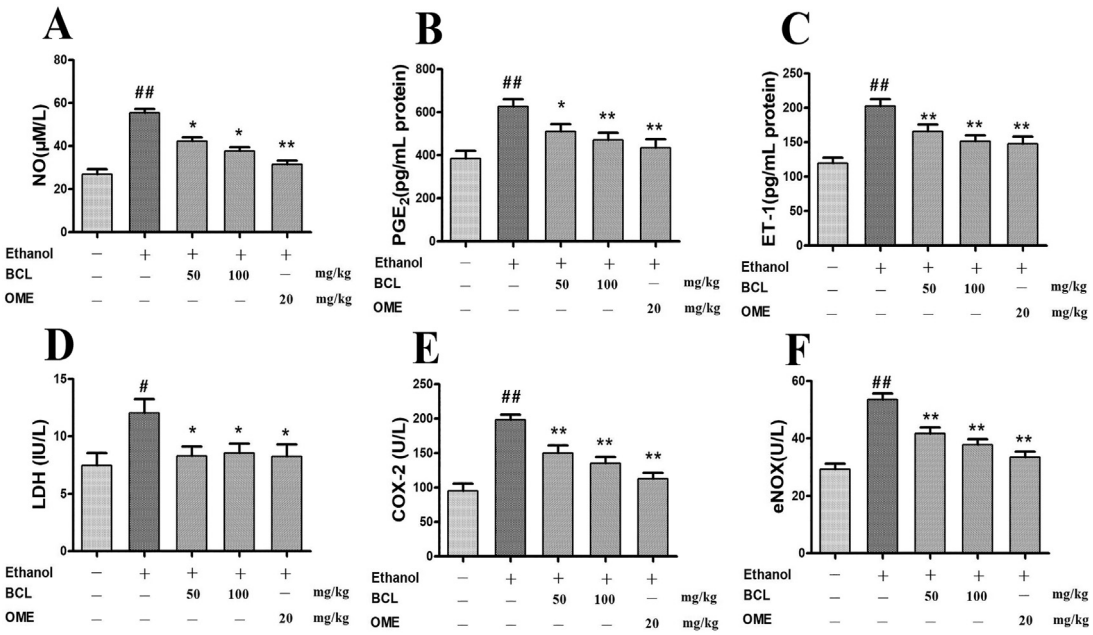

Fig. 3. Baicalin inhibited the inflammatory mediators and NF-κB transcriptional cytokines. (**A**) NO; (**B**) PGE_2; (**C**) ET-1; (**D**) LDH; (**E**) COX-2; (**F**) eNOX. Data are presented as mean ± SD ($n = 6$). $^{\#\#}P < 0.01$ vs. Control group, $^{\#}P < 0.05$ vs. Control group, $^{**}P < 0.01$ vs. Ethanol group, $^{*}P < .05$ vs. Ethanol group.

endothelial NO synthase (eNOS) and cyclooxygenase-2 (COX-2) in macrophages or activation of caspase-3 induced apoptosis [14]. Report showed that baicalin suppressed the NF-κB activation in the inflammatory diseases [15]. These inflammatory cytokines were all downstream of NF-κB signaling pathway, and baicalin could regulate the phosphatidylinositol-3-kinases (PI3K)/protein-serine-threonine kinase (Akt) pathway, inhibiting cell growth and inducing apoptosis [16,17]. Therefore, another aim of the study was to investigate the therapeutic mechanism of baicalin via Akt/NF-κB signaling pathway on a rat model of ethanol-induced chronic gastritis.

2. Materials and methods

2.1. Reagents and materials

Baicalin (Purity, 90%) (Yuanye Biological, China); Determination kits for PGE_2, LDH, ET-1, COX-2, eNOS and NO were provided by Sinobest Biotech (Shanghai, China); Enzyme-linked immunosorbent assay (ELISA) kits for IL-8, IL-1β and TNF-α were obtained from Sinobest Biotech (Shanghai, China); Primary antibodies against p-Akt, Akt, IκBα, p-IκBα, NF-κBp65 and NF-κBp-p65 were provided by Sino Biological (Beijing, China); Omeprazole (Luoxin, China).

2.2. Animals

Male Sprague Dawley (SD) rats (180–200 g) were purchased from B &K laboratory Animal, Co., Ltd. The animals were housed in a controlled environment (12 h light and 25 °C, relative humidity of 60%–65%). All animal experimental procedures were carried out in accordance with the Institutional Animal Committee of Shanghai University of Traditional Chinese Medicine (Permit No.PZSHUTCM190308019).

2.3. Establishment of ethanol-induced gastritis

Forty rats were randomly assigned to five groups as follows ($n = 8$): normal group, model group (rat model of ethanol-induced gastritis), high-dose baicalin-treated group (100 mg/kg), medium-dose baicalin-treated group (50 mg/kg), Omeprazole (OME) group (20 mg/kg). Gastritis was induced with 56% ethanol according to an established procedure. After 12 h fasting, the rats received 56% ethanol (8 g/kg) through gastrogavage twice a week (every Tuesday and Friday). Four weeks later, the chronic gastritis model was fully established. In the model therapy groups, baicalin (BCL), or Omeprazole (OME) were orally administered. Normal and model groups received saline following the same protocol. All the therapeutics were administered once daily for seven days. On the eighth day, all rats were anaesthetized using 2% pentobarbital sodium (0.3 ml/100 g) via intraperitoneal injection and the stomach was immediately taken after blood was gathered from the abdominal aorta.

2.4. The gross anatomy of gastric mucosa and histopathological analysis

The rats of stomach were carefully harvested, cleaned with saline and blotted with filter paper. The length and width of the gastric mucosa area was detected by a vernier caliper. According to Guth standard

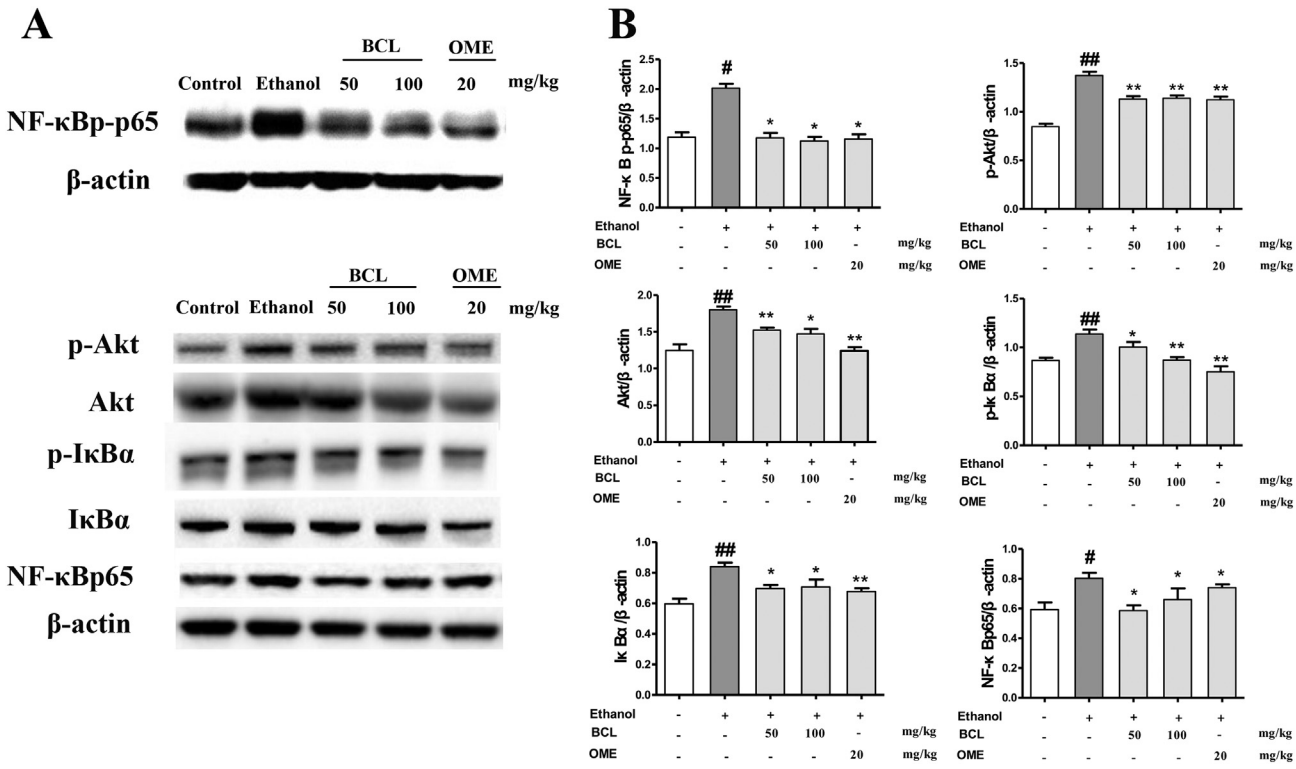

Fig. 4. Effects of baicalin on the activation of signaling enzymes regulating Akt/NF-κB translocation. (**A**) Proteins levels of Akt, p-Akt, IκBα, p-IκBα, NF-κBp65 and NF-κBp-p65 in the rat stomach were analyzed by western blotting. (**B**) The relative intensity of bar chart demonstrated quantification of proteins, the bars showed means ± SEM of three independent experiments. $^{\#\#}P < 0.01$ vs. Control group, $^{\#}P < 0.05$ vs. Control group, $^{**}P < 0.01$ vs. Ethanol group, $^{*}P < 0.05$ vs. Ethanol group.

[18], gastric mucosa ulcer index (UI) was performed as follows: spot erosion (a score of 1), erosion length < 1 mm (a score of 2), erosion length between 1 and 2 mm (a score of 3), erosion length between 2 and 3 mm (a score of 4), and > 3 mm (a score of 5), in addition, the erosion width > 1 mm, the score was doubled, respectively.

According to operating procedure, the stomach tissues close to the gastric antrum were fixed in a 4% paraformaldehyde for 24 h, sectioned and embedded in paraffin. Sections (5 μm thickness) were deparaffinized, stained with hematoxylin and eosin (H&E), then detected under a light microscope to analyze gastric mucosa damage. The degree of gastritis was evaluated according to the established standards: normal gastric mucosa (a score of 1), mucosal epithelial cell injury (a score of 2), damage involving glandular cells (a score of 3), infiltration of lymphocytes, edema, and congestion (a score of 4), a 1 cm length in every slice was observed, and the cumulative score of every slice was calculated.

2.5. ELISA assay

The rats serum was separated from 4 mL of blood by centrifugation at 4 °C for 15 min at 3000 ×*g*. Serum levels of IL-2, IL-8, and TNF-α were measured with ELISA kit strictly following the manufacturer's instructions.

2.6. Detection of NO, ET-1, PGE_2, LDH, COX-2 and eNOS levels in serum

Serum level of NO was carried out by a chemiluminescence. Each supernatant was mixed with Griess reagent for 10 min. The absorbance values were detected at 450 nm, and the levels of NO were measured with the standard curve of sodium nitrite. Detection of Endothelin-1 (ET-1) level was carried out by a ET assay kit. The levels of PGE_2 in serum were detected by enzyme immunoassay using a commercial kit. COX-2 activity was detected using an assay kit. The content of eNOS in serum was detected by eNOS EIA kit, and LDH activity levels were carried out by a LDH kit.

2.7. Western blot analysis

The gastric antrum tissues were lysed with lysis buffer, and the protein yield was detected by BCA protein assay, then total protein extract of the gastric antrum samples were separated by SDS-polyacrylamide gel electrophoresis using 10% gel and then transferred to PVDF membranes, then blocked with 5% non-fat dried-milk in TBST buffer, and incubated with antibodies (1:1000) against Akt, p-Akt, IκBα, p-IκBα, NF-κBp65, NF-κBp-p65 and β-actin at 4 °C overnight. Subsequently, membranes incubated with the secondary antibodies. Images were detected by the enhanced chemiluminescence (ECL) detection system. The chemiluminescence signals were analyzed with Alpha Innotech software. The quantitative method of protein strips was detected by the gray density, and the densities of the protein strips were normalized using β-actin.

2.8. Immunofluorescence staining

Paraffin sections were dewaxed and dehydrated by gradient alcohol, washed three times using PBS (37 °C), and fixed with 4% paraformaldehyde (in PBS) for 30 min. After 1 h in blocking buffer, the paraffin sections were incubated at 4 °C overnight with anti-NF-κBp65 (1:100), washed for 3 times in PBS, then labeled with a fluorescein isothiocyanate (FITC)-IgG antibody, washed in PBS 3 times and 4′,6-diamidino-2-phenylindole (DAPI) stained for 2 min. Tissue sections were labeled with a fluorescein isothiocyanate at room temperature for 1 h after three 5 min washes in PBS, and examined under a fluorescence microscope.

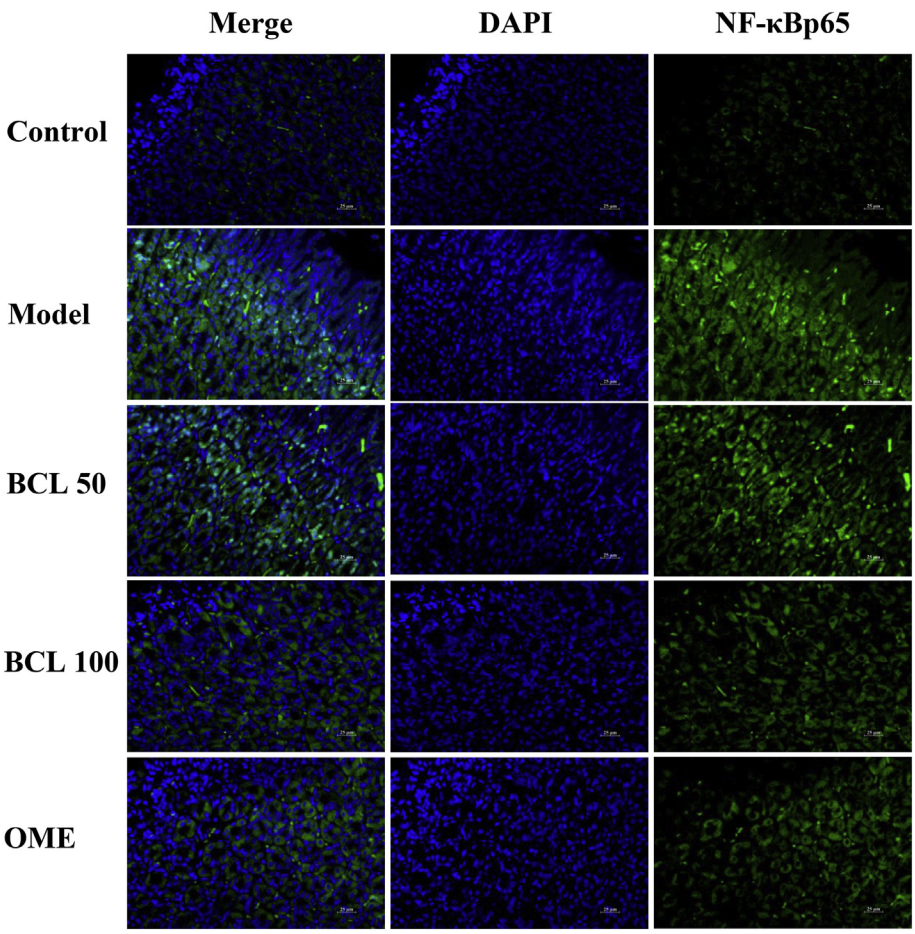

Fig. 5. Baicalin inhibited nucleocytoplasmic translocation of NF-κBp65, the localization of NF-κBp65 in the cytoplasm and nuclear was detected by immunofluorescence staining. NF-κBp65 was stained with anti-NF-κBp65 antibody (coloured in green). The nuclear protein was stained with DAPI (blue). Merge represented the combined image of NF-κBp65 fluorescence and nuclear staining. Images of stomach tissues were performed on a microscope at ×200 magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.9. Molecular docking analysis

To further analyze the way in which baicalin interacts with target proteins of pathway, the interactions between baicalin and IκBα, Akt, NF-κBp65 were measured by Discovery Studio 3.0 software. It was meaningful to clarify how baicalin works with these target proteins from the molecular simulation point. The mol2 structure of small molecule compound was obtained from Pub Chem database (https://pubchem.ncbi.nlm.nih.gov/), and the crystal structures of the protein were obtained from PDB database (http://www.rcsb.org/). The entire molecular docking process included preparation of proteins, determination of docking sites, and docking of proteins to small molecules. Firstly, in the protein preparation process, water molecules were removed from the protein structures, as well as the amide moieties in the side chain from the surrounding residues. Secondly, the binding site on proteins were defined and edited. Finally, the structure of baicalin was embed into the binding site and docked in the generated grid by docking ligands. The force of interactions between compounds and proteins, such as conventional hydrogen bond, carbon hydrogen bond, and π-alkyl, were performed in the output results. Docking parameters: docking preferences, high quality; number of hotspots, 100; conformation method, fast; docking tolerance, 0.25. The labeled interaction site and a corresponding docking score will be showed once the small molecule successfully docks with the protein.

2.10. Statistical analysis

The results were presented as the mean ± standard error of the mean (SEM). Besides, the differences between groups are analyzed by One-Way ANOVAs, and $P < 0.05$ was thought to be significant.

3. Results

3.1. Baicalin attenuated ethanol-induced gastritis in rats

From the analysis of gross anatomy of gastric mucosa, as compared with rats in the normal group, the rats from model group demonstrated the erosion, ulcers, and bleeding on the gastric mucosa. The UI scores were increased. Meanwhile, the gastric mucosal damage in baicalin-treated group (50 mg/kg, 100 mg/kg) was significantly reduced, and the erosion length of gastric mucosal was notably decreased, as well as lower UI scores. The symptoms of gastric mucosal damage were alleviated from the BCL-treatment groups as compared with those from the model group (Fig. 1A–B).

HE staining demonstrated the infiltration of immune cells in the model group, and baicalin significantly reduced this infiltration (Fig. 1C). Extensive lymphocyte infiltration was observed in the intrinsic, submucosal and serosal layer in rats from the model group. Edema and congestion were apparent in the submucosal and intrinsic layers, whose inflammatory scores were increased (Fig. 1D). However, the gastric surface mucus layer in rats from baicalin-treated groups (50 mg/kg, 100 mg/kg) was intact, and the gastric mucosa contained few lymphocytes and showed less edema, the inflammatory scores were significantly decreased.

3.2. Baicalin reduced the expression levels of inflammatory cytokines in ethanol-induced gastritis rats

IL-8, IL-1β and TNF-α are identified as the crucial cytokines involved in inflammation-related diseases. The production of these inflammatory factors was detected in serum levels. ELISA were carried out to detect the levels of IL-8, IL-1β, and TNF-α (Fig. 2A–C). Exposure

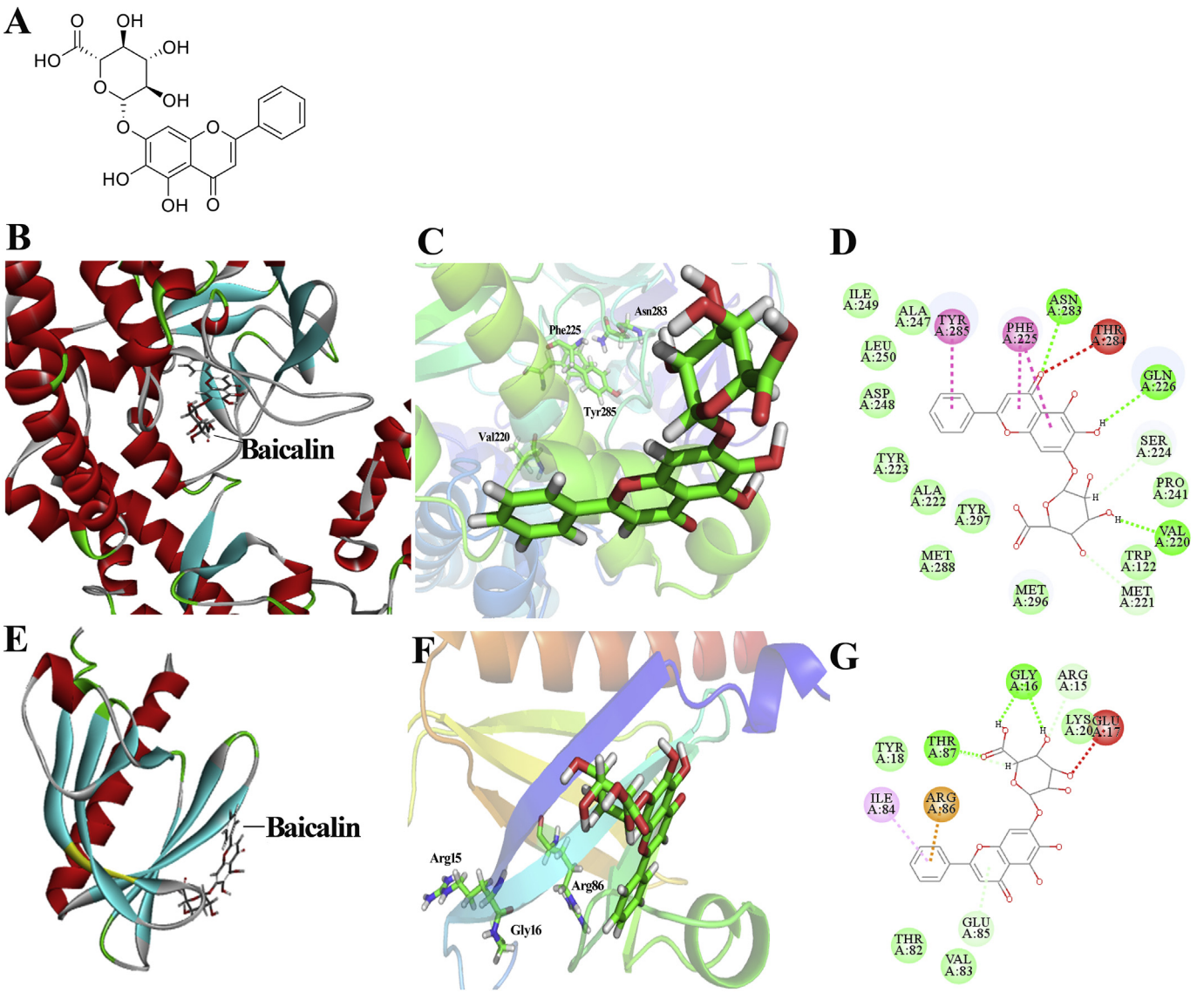

Fig. 6. Effects of baicalin on NF-κBp65 and Akt through molecular docking analysis. (**A**) The chemical structure of baicalin. (**B**) Front view of the docking mode of baicalin in the binding site of NF-κBp65. (**C**) Representative amino acid residues surrounding baicalin in the binding pocket of NF-κBp65. (**D**) Two-dimensional interaction map of baicalin and NF-κBp65. (**E**) Front view of the docking mode of baicalin in the binding site of Akt. (**F**) Representative amino acid residues surrounding baicalin in the binding pocket of Akt. (**G**) Two-dimensional interaction map of baicalin and Akt.

to 56% ethanol significantly increased the levels of IL-8, IL-1β and TNF-α in rats. However, as compared with model group, BCL (50 mg/kg and 100 mg/kg) decreased this increased IL-8 expression. Meanwhile, BCL could down-regulated IL-1β and TNF-α expression, ameliorating the symptoms of ethanol-induced gastritis.

3.3. Effect of baicalin on the inflammatory mediators

PGE_2 and NO were the inflammatory mediators. The activity of NO was increased in the ethanol-induced gastritis group. As compared with the model group, NO levels were significantly reduced by baicalin and OME. As compared with normal group, the activities of PGE_2 were up-regulated in the model group, while, the levels of PGE_2 were notably reduced by baicalin and OME ($P < 0.05$). Meanwhile, baicalin down-regulated NO and PGE_2 expression (Fig. 3A–B).

3.4. Effect of baicalin on the levels of ET-1 and LDH

ET-1 is a potent vasoconstrictor that involved in the gastric mucosal injury process, which was considered to be the strongest endogenous transmitter [19]. It was demonstrated in Fig. 3C that the levels of ET-1 were increased in rats from model group ($P < 0.01$), which indicated that ethanol could induced the production of the vascular regulator. Baicalin and OME could significantly inhibit the levels of ET-1. Meanwhile, exposure to 56% ethanol increased the activities of LDH, reports showed that LDH takes part in glycolysis enzyme biological process [20,21]. It was shown that ethanol interfered with the process of glucose metabolism in stomach tissue. However, baicalin (50 mg/kg and 100 mg/kg) also significantly down-regulated LDH expression (Fig. 3D) ($P < 0.05$).

3.5. Effect of baicalin on the NF-κB transcriptional activation

As compared with normal group, COX-2 was notably enhanced in rats from the model group ($P < 0.01$), while were notably blocked by baicalin (50 and 100 mg/kg) (Fig. 3E). Meanwhile, eNOS could be induced by NF-κBp50, which were involved in the NF-κB signaling pathway. In Fig. 3F, as compared with normal group, eNOS was notably

enhanced in the model group ($P < 0.01$), while were notably inhibited by BCL (50 and 100 mg/kg).

3.6. Baicalin treatment inhibited Akt/NF-κB activation in ethanol-induced gastritis

In order to investigate whether baicalin was able to modulate Akt/NF-κB signaling, the amounts of Akt, p-Akt, IκBα, p-IκBα, NF-κBp65 and NF-κBp-p65 were assessed. Of these, Akt is a marker of PI3K activation, and the phosphorylation of Akt participated in IκBα phosphorylation. From the results of western blot (Fig. 4A), a significant increase in Akt phosphorylation in the gastric antrum tissue of ethanol-induced rats was observed and this increase was reduced when rats were treated with baicalin (50 mg/kg and 100 mg/kg). Additionally, the Akt downstream cascade, NF-κB, was also investigated and the expression level was demonstrated in Fig. 4A. As compared to normal control group, the phosphorylated IκBα and NF-κBp65 were increased in ethanol group, oral treatment with BCL (50 mg/kg and 100 mg/kg) significantly suppressed the levels of p-Akt, Akt, IκBα, p-IκBα, NF-κBp65 and NF-κBp-p65. Immunofluorescence results demonstrated that NF-κBp65 entered the nucleus in ethanol-treated group more than in normal group. However, baicalin (50 mg/kg and 100 mg/kg) suppressed the fluorescence intensity of NF-κBp65, so ethanol-induced NF-κBp65 translocation to the nucleus was inhibited by baicalin, which indicated that the activation of NF-κB signaling pathway was suppressed by BCL (Fig. 5).

3.7. Docking of baicalin at the potential protein active site

To analyze the interaction force between baicalin and the target proteins, including IκBα, NF-κBp65 and Akt. However, IκBα has no corresponding ligand, so the molecular docking test cannot be performed. The chemical structure of baicalin was shown in Fig. 6A. The docking mode of baicalin in the biding site of NF-κBp65 (PDB: 3QXY) was illustrated in Fig. 6B–D. The three-dimension and two-dimension interaction schematic diagrams showed that the benzene rings of chromone of baicalin formed π-π interactions with Phe225 and Tyr285, and the carbonyl group of chromone with Asn283 formed H-bond. The glycosyl of baicalin formed a conventional hydrogen bond with Val220. The docker interaction energy between baicalin and NF-κBp65 was 34.73 kcal/mol. It showed that baicalin could combine to the activity site of NF-κBp65. For the docking mode between baicalin and Akt (PDB: 1UNQ) (showed in Fig. 6E-G), the glycosyl of baicalin could form a conventional hydrogen bond with Gly16 and Arg15, and its benzene rings formed strong ionic bond with Arg86. The docker interaction energy between baicalin and Akt was −83.15 kcal/mol. These results showed the affinity between baicalin and the active site of Akt. Based libdock scores, the libdock scores of baicalin with Akt, baicalin with NF-κBp65 were 114.331 and 149.999, respectively. Therefore, NF-κBp65 had a higher affinity with baicalin.

4. Discussion

Ethanol-induced chronic gastritis is a chronic and inflammatory disease along with clinical manifestations, such as the damage of gastric mucosa, loss of appetite, weight loss, abdominal pain and diarrhea [22]. However, current therapy applied for the ethanol-induced chronic gastritis is limited and adverse reactions remains a main issue in the clinic [23,24]. Moreover, it is urgent to uncover the mechanisms of ethanol-induced chronic gastritis and seek better therapeutic approaches. Baicalin, is a bioactive compound from Scutellaria Radix with pharmacological activities including anti-inflammatory bioactivities, immunomodulatory and anti-macrobial functions [25,26]. Previous study demonstrated that baicalin ameliorated the inflammatory cells infiltration and performed well effects on gastritis [27]. Interestingly, in our study, it was meaningful to find that baicalin demonstrated significant therapeutic effects on ethanol-induced gastritis. Firstly, baicalin treatment notably reduced some indices including UI scores and inflammatory scores, which significantly alleviated the degree of the gastric mucosal damage. Besides, it was also found that baicalin markedly reduced the production of IL-8, IL-1β and TNF-α. Furthermore, baicalin inhibited the levels of Akt, IκBα and NF-κBp65, as well as inhibited the levels of phosphorylated Akt, IκBα and NF-κBp65, which indicated that baicalin suppressed the Akt/NF-κB pathway activation.

Additionally, the levels of inflammatory regulators, such as NO, PGE_2, eNOX and COX-2, were examined similar to the report that baicalin could perform an anti-inflammatory effect by reducing the expression level of PGE_2, NO, eNOX and COX-2 [28]. Furthermore, COX-2 and eNOS were involved in the NF-κB transcriptional activation, they were involved in the process of some certain inflammatory diseases [29]. Previous study demonstrated that microcirculatory disorder could cause the gastric mucosal barrier injury, as well as increasing the levels of ET-1 and NO [30,31]. PGE_2 is an important substance mediated by inflammation, which is involved in the metabolic pathway of arachidonic acid. Report showed that reducing of PGE_2 and COX-2 expression could suppress the acute pharyngitis in mice [32]. In our study, the levels of ET-1, PGE_2, NO, eNOX and COX-2 were decreased by baicalin, which ameliorated the inflammatory effects of ethanol gastritis.

ET-1 is the strongest vasoconstrictor in the body, which could reduce gastric mucosal injury [33]. Interestingly, it has been reported that the COX-2 inhibitor, rofecoxib, can reduce the damage of EtOH/HCl gastritis [34]. It was speculated that LDH may be a diagnostic marker for ethanol gastritis. Ethanol gastritis has a metabolic pathway of tricarboxylic acid aerobic metabolism and anaerobic glycolysis, LDH was increased in chronic gastritis, carcinoma and intestinal metaplasia [35,36]. It may indicate that baicalin can reduce the injury of gastric mucosa by regulating the energy metabolism of gastric tissue. Therefore, baicalin took part in the regulation of anti-inflammatory factors by blocking NO and PGE_2, while inhibiting the levels of COX-2 and eNOX. This result indicated that baicalin also achieved a gastric acid-protective mucosal layer by increasing the thickness or by retaining subepithelial microvascular integrity, and these effects require further investigation of how baicalin acts in these processes.

Furthermore, immunoblotting study has revealed changes in the expression levels of upstream and downstream proteins on Akt/NF-κB pathway, these findings supported the hypothesis that baicalin may modulate this signaling pathway. It is an important cellular signaling cascade that is widespread in the inflammatory response [37]. For instance, the expression level of NF-κBp65, which is a crucial submit of NF-κB, was markedly inhibited by baicalin. Furthermore, the phosphorylated NF-κB-signaling molecules, including Akt and IκBα, were also suppressed by baicalin. The previous study demonstrated that Akt is the target protein of PI3K-Akt signaling following stimulation and primes NF-κB activation [38]. PI3K-dependent Akt phosphorylation is involved in the inflammatory pathways in gastritis [39]. Akt is the direct downstream protein of PI3K [40]. Report showed that the phosphorylation of Akt protein participated in anti-gastritis, NF-κB involved in the cascade of Akt signaling, IκBα phosphorylation can be promoted by phosphorylation of Akt protein [41]. The NF-κB transcription factor family is a key response process of inflammation and is considered as a therapeutic target in immune defense systems by inhibiting activated NF-κB [42]. Besides, MAPK and Akt are also involved in many pro-inflammatory genes [43]. NF-κB is a downstream protein in the PI3k-Akt signaling pathway, it could be activated by the phosphorylated IκK kinase, which inducing the degradation of IκB [44]. Report showed that non-activated NF-κB binds to the IκBα protein in the cytoplasm, leading the activation of NF-κB. When IκBα is phosphorylated, ubiquitinated and subsequently degraded, then NF-κBp65 translocates to the nucleus and transcribes the target gene [45,46]. The activation of NF-κBp65 by different stimuli causing the induction of numerous genes encoding proinflammatory cytokines and enzymes, including COX-2, IL-8, IL-1β

and TNF-α, however, the activation of NF-κBp65 was inhibited by baicalin, which will contribute to the inhibition on the release of inflammatory factors.

5. Conclusions

This study is the first time demonstrated that baicalin reduced inflammatory mediators (NO and PGE_2), decreased the levels of metabolic regulator (LDH), vasoconstriction factor, ET-1, and suppressed the NF-κB activation, then decreased the levels of inflammatory cytokines, including IL-8, IL-1β and TNF-α. In conclusion, baicalin may be a potential molecule on the treating alcoholic gastritis.

Author contributions

WJ designed experiments and wrote the manuscript; KL performed experiments and analyzed the results; RA designed the main experiments; XW revised and approved the submitted version.

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Declaration of competing interest

All authors declare no conflicts of interest.

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