Heliyon 9 (2023) e21224

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Atractylenolide III ameliorated reflux esophagitis via PI3K/AKT/NF-κB/iNOS pathway in rats

Xianzhe Si [a,1], Weijie Lin [a,1], Zhiyao Chen [a,*], Jie Xu [a], Wenbo Huang [a], Feng Chen [a], Jianqing Lin [b], Zhijun Huang [a,**]

[a] Department of Gastrointestinal & Esophageal Surgery, The 2nd Affiliated Hospital of Fujian Medical University, Quanzhou, China
[b] Department of Oncology, The 2nd Affiliated Hospital of Fujian Medical University, Quanzhou, China

ARTICLE INFO

Keywords:
Atractylenolide III
Reflux esophagitis
PI3K/AKT/NF-κB/iNOS pathway
Rats

ABSTRACT

Reflux esophagitis (RE), an esophageal inflammation caused by reflux of gastric contents, often damages the lower esophagus, seriously affecting the quality of life of patients. This study aims to investigate the therapeutic effects and underlying molecular mechanisms of atractylenolide III (ATL III) on RE model rats. In this research, the RE rat model is established sequentially following hemipyloric ligation, cardia transection, and hydrochloric acid perfusion. Further, the RE-induced rats are intragastrically administrated with ATL III (0.6, 1.2, and 2.4 mg/kg/D) for 28 days to evaluate ATL III therapeutic effects. To study the molecular mechanism, RE rats are treated with a phosphoinositide-3 kinase (PI3K) agonist (740 Y–P) combined with ATL III. The histopathological changes in the esophagus are eventually observed by hematoxylin & eosin (H&E) staining. In addition to changes in gastric pH and levels of reactive oxygen species (ROS), enzyme-linked immunosorbent assay (ELISA) and Western blot analyses are used to detect the expression levels of tumor necrosis factor-α (TNF-α, mmol/L), interleukin (IL)-8, IL-6, IL-1β in the esophageal tissues. As a result, the lesions in the esophageal tissues of RE rats are alleviated, decreasing the macroscopic observation scores of the esophageal mucosa after ATL III treatment,. The experimental results indicated significantly increased pH value of the gastric contents and reduced ROS, thiobarbituric acid reactants (TBARS), TNF-α, IL-8, IL-6, and IL-1β levels, as well as expression levels of p-PI3K, *p*-AKT, iNOS, and nuclear NF-κB proteins in esophageal tissues. In conclusion, the study indicated that ATL III could efficiently treat RE in rats by inhibiting oxidative stress and inflammatory damage through the PI3K/AKT/NF-κB/iNOS pathway.

1. Introduction

Reflux esophagitis (RE) is referred to as an esophageal inflammation caused by lower esophageal injury, leading to the reflux of gastric contents. The concomitant reflux of gastric contents often causes severe inflammation and ulceration, substantially destroying the normal esophageal mucosa and tissues. This gastric reflux disease is characterized by a burning sensation in the chest (sometimes called heartburn) and nausea after a meal [1,2]. However, the exact mechanism underlying the development of the RE condition

* Corresponding author.
** Corresponding author.
E-mail addresses: chenzyaoo@fjmu.edu.cn (Z. Chen), huangzj@fjmu.edu.cn (Z. Huang).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2023.e21224
Received 5 June 2023; Received in revised form 17 September 2023; Accepted 18 October 2023
Available online 23 October 2023

Abbreviations

ANOVA	applying the one-way analysis of variance
ATL III	atractylenolide III
ELISA	enzyme-linked immunosorbent assay
H&E	hematoxylin & eosin
HO-1	heme oxygenase 1
IL:	interleukin
NQO1	nicotinamide adenine dinucleotide phosphate-quinone oxidoreductase 1
Nrf2	nuclear factor erythroid-2-related factor 2
PI3K	phosphoinositide-3 kinase
PPIs	proton pump inhibitors
RE	reflux esophagitis
ROS	reactive oxygen species
SD	Sprague-Dawley
SPF	specific pathogen-free
TBARS	thiobarbituric acid reactants
TNF-α	tumor necrosis factor-α

remains unclear. Accordingly, previous reports indicated that alcohol, smoking, obesity, stress, and other factors were identified as the sources of the RE pathogenesis [3]. In this context, a recent study demonstrated that oxidative stress was a critical pathogenetic factor in RE pathogenesis [4,5]. Due to oxidative stress, hydrogen ions could substantially enrich mucosal tissues and promote the infiltration of reactive oxygen species (ROS) into cells, releasing cytokines and resulting in acidosis, as well as necrosis of esophageal tissues [6]. In another instance, since the overproduction of ROS could cause inflammation, antioxidants have been shown to block free radicals, preventing esophageal mucosal injury [7]. Currently, the clinical management of RE is often based on using gastric acid-lowering anti-secretory therapies, such as proton pump inhibitors (PPIs), antacids, and histamine receptor antagonists. Although inhibiting gastric acid secretion is the mainstay of treatment, the therapeutic efficacy of PPIs against reflux disease is far from a clinical need. In addition, long-term treatment with the above drugs often results in severe undesired complications and various adverse effects in some patients [8,9]. Consequently, searching for traditional Chinese medicines or natural compounds with profound antioxidant activity has attracted increasing attention to treat RE.

Atractylenolide III (ATL III), a sesquiterpenoid isolated from *Rhizoma Atractylodis Macrocephalae*, has been reported to possess various pharmacological activities, including gastroprotective and neuroprotective effects, among others [10,11]. Accordingly, several reported studies also confirmed its excellent anti-inflammatory and antioxidant efficacies. In several instances, ATL III exerted significant ameliorative effects on several diseases (*e.g.,* chronic kidney disease, idiopathic pulmonary fibrosis, and isoflurane-induced neuronal injury in the rat hippocampus). Specifically, ATL III executed its actions by regulating the PI3K/AKT/mTOR or nuclear factor erythroid-2-related factor 2 (Nrf2)/nicotinamide adenine dinucleotide phosphate-quinone oxidoreductase 1 (NQO1)/heme oxygenase 1 (HO-1) signaling pathways and inhibiting oxidative stress and inflammation [12–14]. However, the therapeutic effects and underlying mechanism of ATL III on RE remain unclear.

Considering these aspects, the present study is aimed to investigate the therapeutic effects and underlying molecular mechanistic action of ATL III on RE rats. To explore these attributes, we established the RE model sequentially following hemipyloric ligation, cardia transection, and hydrochloric acid perfusion. After 28 days of ATL III, the pH value of gastric contents, the levels of reactive oxygen species (ROS), and thiobarbituric acid reactants (TBARS) in esophageal tissues were detected. To study the molecular mechanism, RE rats were treated with a phosphoinositide-3 kinase (PI3K) agonist (740 Y–P) combined with ATL III. Further, enzyme-linked immunosorbent assay (ELISA) kits were used to detect the expression levels of tumor necrosis factor-α (TNF-α), mmol/L), interleukin (IL)-8, IL-6, IL-1β, and Western blot analysis for TNF-α, IL-8, IL-6, IL-1β, and PI3K/AKT/NF-κB/iNOS pathway protein expression levels in esophageal tissues. Eventually, the histopathological changes in the esophagus were observed by hematoxylin & eosin (H&E) staining.

2. Materials and methods

2.1. Materials

ATL III and 740 Y–P were obtained from Abmole Bioscience Inc. (Shanghai, China). 1,1,3,3-tetramethoxypropane, thiobarbituric acid, paraformaldehyde, and ELISA kits of TNF-α, IL-8, IL-6, and IL-1β were obtained from Sigma Co. Ltd. (St. Louis, USA). HCl and butanol were obtained from Sinopharm Group Co. Ltd. (Shanghai, China). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was obtained from Beyotime Biotechnology Co. Ltd. (Shanghai, China). All the other chemicals and reagents were obtained from Sigma Co. Ltd. unless otherwise stated.

2.2. Animals

The Sprague-Dawley (SD) rats (n = 90, both sexes, weighing 200 ~ 250 g) were purchased from Shanghai Laboratory Animal Co. Ltd. (SLAC, Shanghai, China). The SD rats were housed in a specific pathogen-free (SPF) grade environment, giving access to a standard diet and water ad libitum with a light/dark environmental cycle of 12 h. The temperature of the animal laboratory room was maintained at 20–25 °C and relative humidity at 50–60 %. All the animal-related experimental protocols were approved by the Institutional Animal Care and Use Committee of 2nd affiliated hospital of FJMU (approval number: FJMU-2021-344) and the whole procedures were conducted in accordance with internationally accepted principles for laboratory animal use and care. It should be noted that all rats were acclimatized for at least 7 days before the start of the experiment.

2.3. Establishment of RE model

The RE rat model was established following the reported procedure [15]. Briefly, animals were kept fasting for 24 h before surgery. Further, the animals were anesthetized using the intraperitoneal injection of 0.4 % sodium pentobarbital. Then, the animals were tethered in a supine position, incised the upper abdomen midline for 3 cm, and ligated the rat's pylorus with a 2-0 nonabsorbable suture. Afterward, the muscle layer was cut vertically upward about 0.7 cm, closed the abdomen by suturing. On day-3 after surgery, rats were treated with 0.1 mol/L HCl by gavage at a dose of 1.0 ml/100 g/D for 7 days. For comparison, the stomach and duodenum of the animals in the sham group were separated by 15 min without ligation of the pylorus and duodenum. Eventually, the gavage of the animals in the sham group was performed with water instead of HCl.

2.4. Drug treatments

To evaluate the therapeutic effect of ATL III on the RE group of rats, the successfully constructed RE model rats were randomly divided into five groups of 10 rats per group: sham-operated (sham), model (RE), and RE + ATL III of 0.6 mg/kg, RE + ATL III of 1.2 mg/kg, as well as RE + ATL III of 2.4 mg/kg, following the reported studies [12,13]. On the one hand, the RE Rats in the treatment groups were intragastrically administered with ATL III according to the doses for 28 days. On the other hand, RE rats in the sham and model (RE) groups were intragastrically administered with an equal volume of normal saline. In addition, to further explore the molecular mechanistic effect of ATL III, rats were randomly divided into four groups: sham (sham), model (RE) group, RE + ATL III of 2.4 mg/kg, RE + ATL III of 2.4 mg/kg + 740 Y–P of 10 mg/kg group. The optimal dose of 740 Y–P in rats was administered following the reported study [16]. Further, the rats in the ATL III-treated and combined treatment groups were intragastrically administered with ATL III or ATL III +740 Y–P by gavage at the divided doses as described above for 28 days. To this end, as mentioned earlier, rats in the sham and model (RE) treatment groups were intragastrically administered with an equal volume of normal saline.

2.5. Esophageal mucosal score

The esophageal mucosal score was recorded following the procedure below. After euthanizing the rats, the esophagus of the treated rats was opened and gently rinsed with 0.9 % sodium chloride. Further, the photographs of the precisely injured areas were captured under a dissecting microscope at a magnification of 3 × . Then, the lesion was scored following the scoring criteria as follows: manifesting as a normal flash mucosa (*score 0*), edematous mucosa with focal bleeding points (*score 1*), multiple erosions with heme attachment (*score 2*), linear ulcers with yellow exudates (*score 3*), and hemorrhagic combined ulcers (*score 4*).

2.6. Gastric content pH determination

The euthanized rats were subjected to ligation of the cardia and pylorus. Then, the whole gastric tissues were removed. After the incision along one side of the gastric greater curvature, the lower gastric contents were eluted with 5 ml of distilled water. The pH value of the gastric content supernatant was determined using a pH meter.

2.7. Detection of TBARS

The TBARS were detected following the reported protocol, using 1,1,3,3-tetramethoxypropane as the standard sample [17]. 30 mg of the extracted esophageal tissue sample was collected and mixed with 1 % phosphoric acid. Further, 0.67 % thiobarbituric acid was added to the above mixture and boiled at 95 °C for 45 min. Then, the butanol was added to the mixture and centrifuged at 3000 rpm for 10 min. Finally, the supernatant was collected, and absorbance values were measured by scanning at 540 nm.

2.8. ROS detection

The ROS levels in the esophageal tissue samples were detected following the DCFH-DA assay. Initially, 20 mg of collected esophageal tissue sample was homogenized by adding 2 ml of radioimmunoprecipitation assay (RIPA) lysis buffer solution (Sigma-Aldrich Co. Ltd.) at 4 °C and centrifuged at 5000 rpm for 10 min at 4 °C to separate the tissue supernatant. Further, DCFH-DA (25 mM) was added to the separated tissue supernatant samples and incubated for 30 min. Finally, the changes in the fluorescence intensity were measured at an excitation wavelength of 486 nm and an emission wavelength of 530 nm.

2.9. Pathological detection

The pathological detection of the esophageal tissue samples was performed by H&E as described below. Initially, the tissue samples were fixed in 4 % paraformaldehyde, embedded in paraffin, and sectioned at a thickness of 5 μm. After deparaffinization, the samples were subjected to H&E stain. Then, the morphological changes of the H&E stained esophageal tissue structures were observed under the bright field state of a light microscope.

2.10. ELISA

Briefly, 30 mg of the esophageal tissue sample was collected and homogenized by adding 2 ml of RIPA lysis solution at 4 °C. Further, the sample was centrifuged at 10000 rpm at 4 °C for 10 min to separate the tissue supernatant. Finally, the corresponding ELISA kits of TNF-α, IL-8, IL-6, and IL-1β were applied and detected their expression levels, following the manufacturer's instructions.

2.11. Western blot assay

The Western blot analysis was performed to determine the expression levels of corresponding proteins in the esophageal tissues. Briefly, 30 mg of preserved esophageal tissue after instant freezing in liquid nitrogen was added with 2 ml of RIPA lysis buffer solution and homogenized at 4 °C. Then, the total protein of liver tissue was extracted following the instructions of the protein extraction kit (Bio-Rad Laboratories, Hercules, USA). The protein concentration was quantified by bicinchoninic acid (BCA) colorimetry. Further, 40 μg of protein sample and 5 × SDS loading buffer (4:1 by volume) were mixed, and the separation was performed by subjecting them electrophoretically to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Further, the protein blots were electrotransferred to PVDF membranes. Then, the membranes were blocked with 5 % non-fat milk for 1 h and incubated with the corresponding primary antibodies, such as p-PI3K (1:1000 dilution), PI3K (1:1000 dilution), *p*-AKT (1:2000 dilution), AKT (1:2000 dilution), NF-κB (1: 1500 dilution), iNOS (1:1500dilution), TNF-α (1: 1000 dilution), IL-8 (1: 1000 dilution), IL-6 (1: 1000 dilution), IL-1β (1: 1000 dilution), β-actin (1: 5000 dilution), and Lamin B1 (1:5000 dilution), overnight at 4 °C. Further, the blots were incubated with the addition of horse radish peroxidase (HRP)-conjugated secondary antibodies (Goat anti-rabbit immunoglobulin, IgG, 1:10000; Goat anti-mouse IgG, 1:10000) for 1 h at 37 °C. Finally, the blots were visualized by electrochemiluminescence (ECL) substrate and the results were quantitatively analyzed with Image J software. The integrated absorbance values of the Western blot bands were expressed as the ratio of the integrated absorbance to the phosphorylation rate or expression level of the proteins.

2.12. The protocol of the research

The protocol of the research was be showed in Fig. 1.

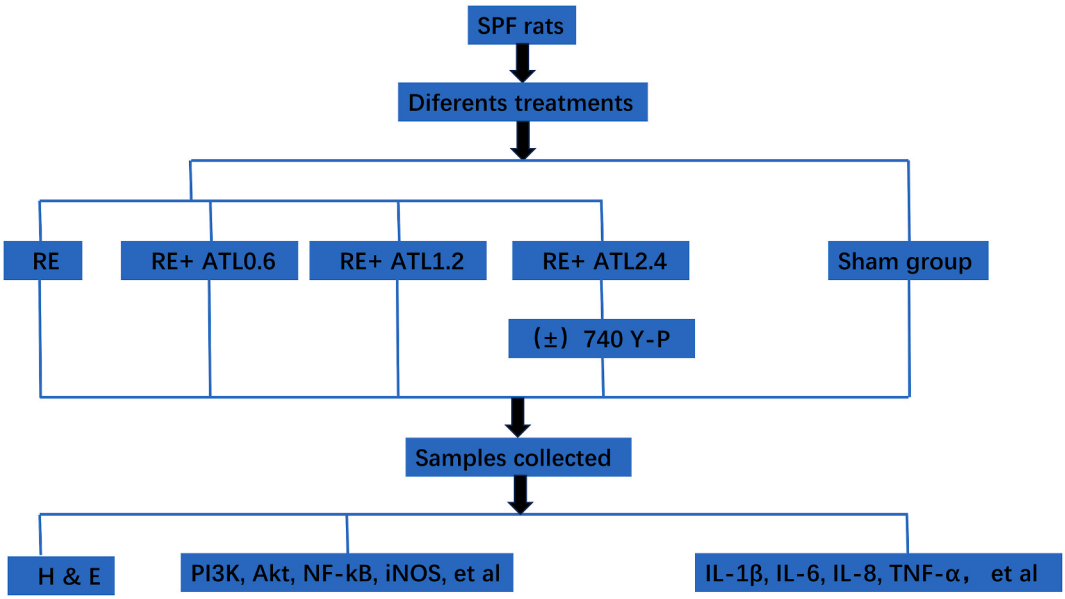
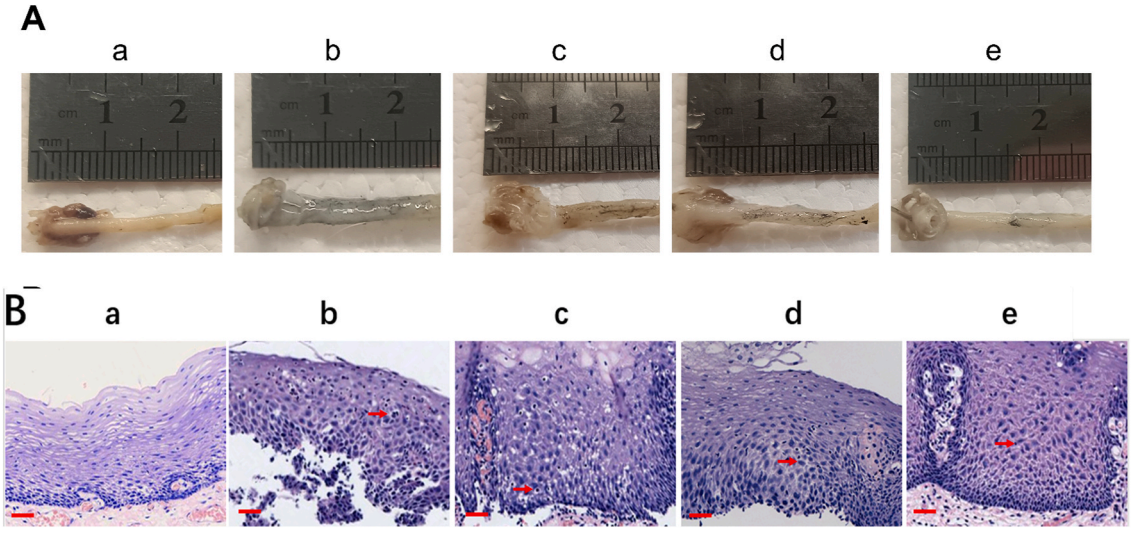
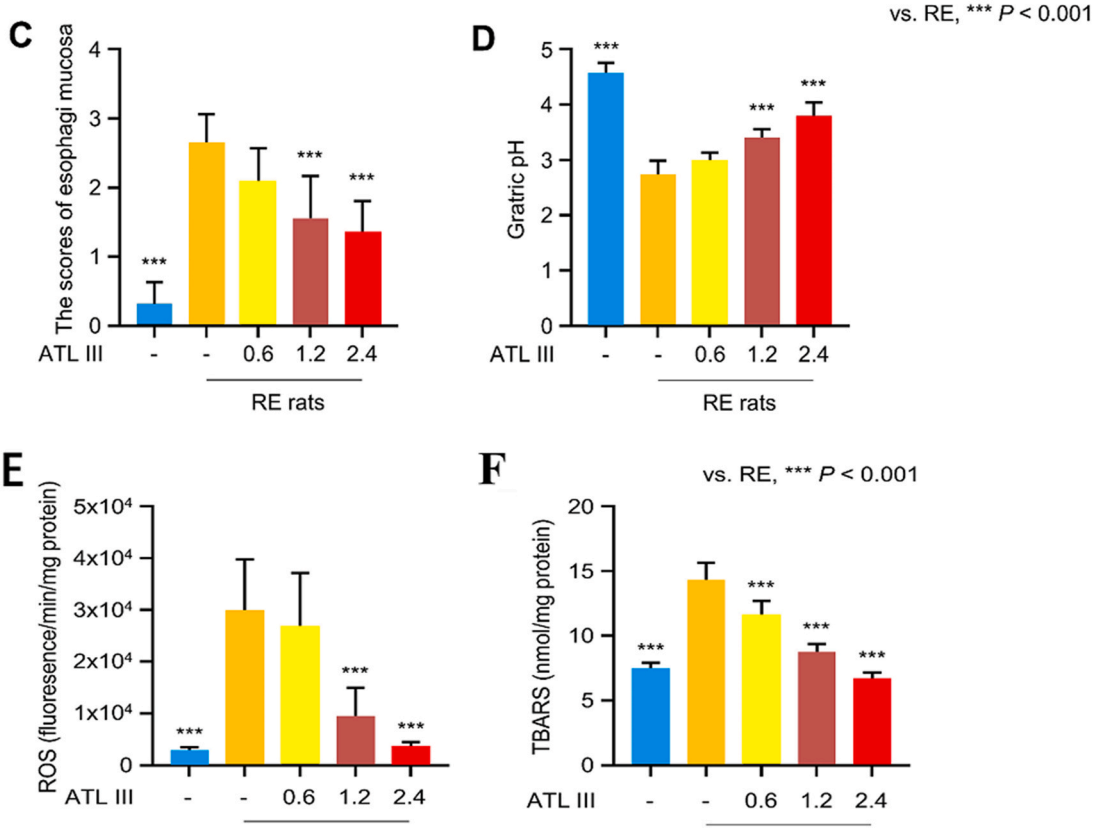


Fig. 1. The protocol of the research.

a: sham
b: RE
c: RE+0.6 mg/kg ATL III
d: RE+1.2 mg/kg ATL III
e: RE+2.4 mg/kg ATL III





(caption on next page)

Fig. 2. ATL III attenuates esophageal injury in RE rats A) Images show the esophagus of rats after RE establishment and ATL III treatment in samples of a) sham group, b) RE group, c) ATL III (0.6 mg/kg), d) ATL-III 1.2 mg/kg, and e) ATL III 2.4 mg/kg treated groups. **B)** H&E stained images of the sectioned esophagus tissues of rats after RE establishment and ATL III treatment. a) sham group, b) RE group, c) ATL III (0.6 mg/kg) d) ATL-III 1.2 mg/kg, and e) ATL III 2.4 mg/kg treated groups. Arrow marker: lymphocyte infiltration. **C)** The graph represents the esophageal mucosal injury score after RE establishment and ATL III treatment. **D)** The graph indicates the gastric pH changes after RE establishment and ATL III treatment. *** indicates $P < 0.001$ vs. RE group. **E)**The graphs present the ROS and **F)** TBARS levels after various treatments of ATL-III group. *** indicates $P < 0.001$ vs. RE group.

2.13. Statistical analysis

The data that met the homogeneity of variance were expressed as mean ± standard deviation (X ± s). Using the SPSS v19.0 software, the experimental data were analyzed by applying the one-way analysis of variance (ANOVA) to compare the differences among groups, followed by Tukey's method for pairwise comparisons between groups, considering $P < 0.05$ as statistically significant. On the one hand, the scoring results of esophageal mucosa were analyzed by the Kruskal-Wallis H test. On the other hand, the Mann-Whitney *U* test was used for pairwise comparison between groups, considering $P < 0.05$ as statistically significant.

3. Results

3.1. ATL III attenuates esophageal injury in RE rats

The macroscopic observations and H&E staining images showed no reflux-induced esophageal lesions in the esophagus of rats in

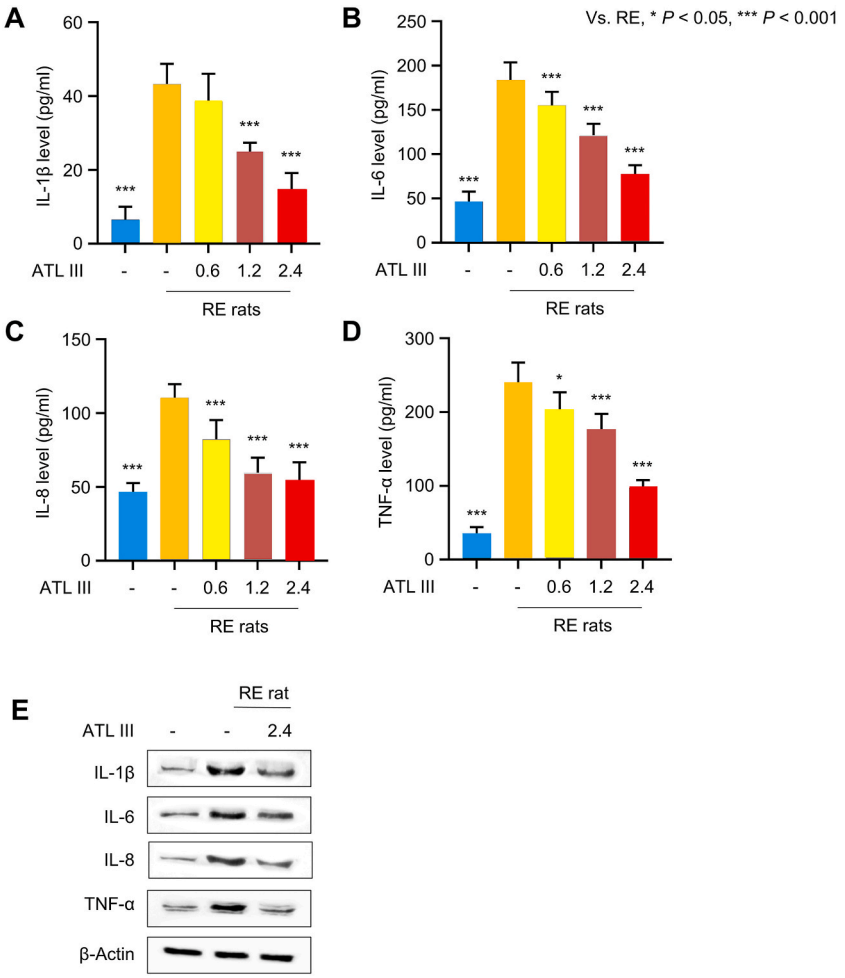
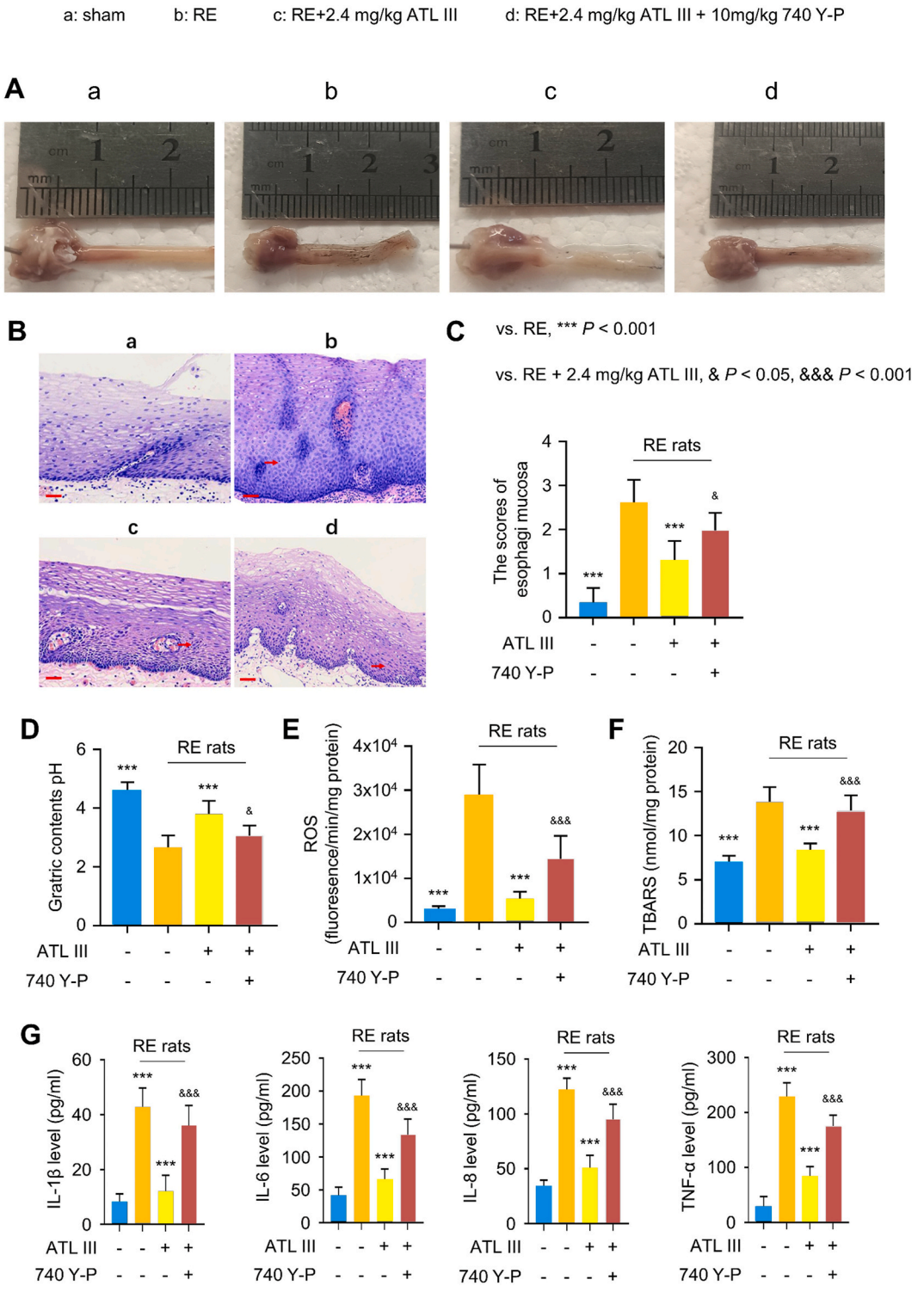


Fig. 3. ATL III attenuates esophageal inflammation in RE rats. The graphs show the levels of various inflammatory indicators, **A)** IL-1β, **B)** IL-6, **C)** IL-8, and **D)** TNF-α. **E)** The Western blot analysis indicates the expression levels of various IL-1β, IL-6, IL-8, and TNF-α proteins against β-Actin as internal standard at the 2.4 mg/kg dose of ATL III. * indicates $P < 0.05$ vs. RE group, and *** represents $P < 0.001$.

sham group. In contrast, reflux-induced esophageal lesions were observed in the esophageal mucosa of RE rats, such as ulceration, massive inflammatory cell infiltration, expansion of epithelial gap, thickening of the basal cell layer of squamous epithelium, and lengthening of the papillae of the lamina propria. Further, the esophageal mucosa of the ATL III-treated rats showed significant changes compared to the RE treatment group in terms of the infiltration of inflammatory cells, expansion of epithelial cell gaps,



(caption on next page)

Fig. 4. ATL III inhibits PI3K/AKT/NF-κB/iNOS pathway. A) Images show the esophagus of rats after treatment with a) sham group, b) RE group, c) RE+2.4 mg/kg of ATL III, d) RE+2.4 mg/kg of ATL III +10 mg/kg of 740 Y–P. **B)** H&E stained images of the sectioned esophagus tissues of rats after treatment with a) sham group, b) RE group, c) RE+2.4 mg/kg ATL III, d) RE+2.4 mg/kg of ATL III +10 mg/kg of 740 Y–P. Arrow marker: lymphocyte infiltration. **C)** The graph represents the esophageal mucosal injury score after treatment with ATL III +10 mg/kg of 740 Y–P in RE rats. **D)** The graph signifies the gastric content pH after treatment with ATL III +10 mg/kg of 740 Y–P in RE rats. **E)** The image shows the ROS levels after treatment with ATL III +10 mg/kg of 740 Y–P in RE rats. **F)** The image shows the TBARS levels after treatment with ATL III +10 mg/kg of 740 Y–P in RE rats. **G)** The image shows the expression levels of various inflammatory indicators (TNF-α, IL-8, IL-6, and IL-1β) after treatment with ATL III + 10 mg/kg of 740 Y–P in RE rats. ** indicates $P < 0.01$ and *** represents $P < 0.001$ vs. RE. $^{\&}$ presents $P < 0.05$ and $^{\&\&\&}$ signifies $P < 0.001$ vs. RE + 2.4 mg/kg ATL III.

thickening of the basal cell layer of squamous epithelium, and lengthening of the papilla of the lamina propria (Fig. 2A and B). Consistent with the results of pathological detection by H&E staining, the damage scores of the esophageal mucosa of rats were observed, in which the RE group showed a significantly higher score than the sham group. In contrast, the ATL III treatment dose-dependently decreased the esophageal mucosal injury score compared with the RE group, in which 1.2 and 2.4 mg/kg doses showed significantly improved scores compared to the 0.6 mg/kg (Fig. 2C). Further, the RE rats showed decreased gastric content pH value compared to the sham group, and ATL III treatment gradually showed an increase in the pH value in the RE rats with an increase in its concentration (Fig. 2D). These results indicated that ATL III showed substantial therapeutic effects on esophageal injury in RE rats, attenuating the esophageal injury in RE rats.

Furthermore, the therapeutic potential of ATL III was explored by measuring the oxidative stress mechanistically. It was observed from the experimental results that the ROS and TBARS levels were significantly increased in the esophageal tissues of rats in the RE group compared with the sham group. Further, the ATL III dose-dependently decreased ROS production (Fig. 2E) and TBARS levels (Fig. 2F) in rat esophageal tissues compared with the RE group, indicating the alleviation of esophageal oxidative stress in RE rats. These results suggested that relieving oxidative stress could be one of the reasons for the actions of ATL III against RE in rats.

3.2. ATL III attenuates esophageal inflammation in RE rats

Similar to the oxidative stress results in RE rats, as depicted in Fig. 3, the ELISA assays of TNF-α, IL-8, IL-6, and IL-1β levels in the rat esophageal tissues of the RE group showed that the levels of the notified inflammatory mediators were significantly increased compared to the sham group. Compared with the RE group, the levels of the above-mentioned inflammatory cytokines in the esophageal tissues of rats were decreased in the ATL III treatment group (Fig. 3A–D). Consistent with the ELISA findings, the Western blot analysis showed that TNF α, IL-8, IL-6, and IL-1β protein expression levels were markedly increased in the esophageal tissues of rats of the RE group than the sham group. Further, the expression levels of the above proteins in the esophageal tissues of rats in the ATL III treatment group (2.4 mg/kg) group were significantly decreased compared to the RE treatment group (Fig. 3E).

3.3. ATL III ameliorated reflux esophagitis via PI3K/AKT/NF-κB/iNOS pathway in rats

The Western blot analysis showed that p-PI3K, *p*-AKT, iNOS, and nuclear NF-κB protein expression levels were markedly increased in the esophagus of the RE rats compared with the rats in sham group. Together, our results suggested that the amelioration of esophageal injury in RE rats by ATL III might be related to the inhibition of the PI3K/AKT/NF-κB/iNOS signaling pathway.

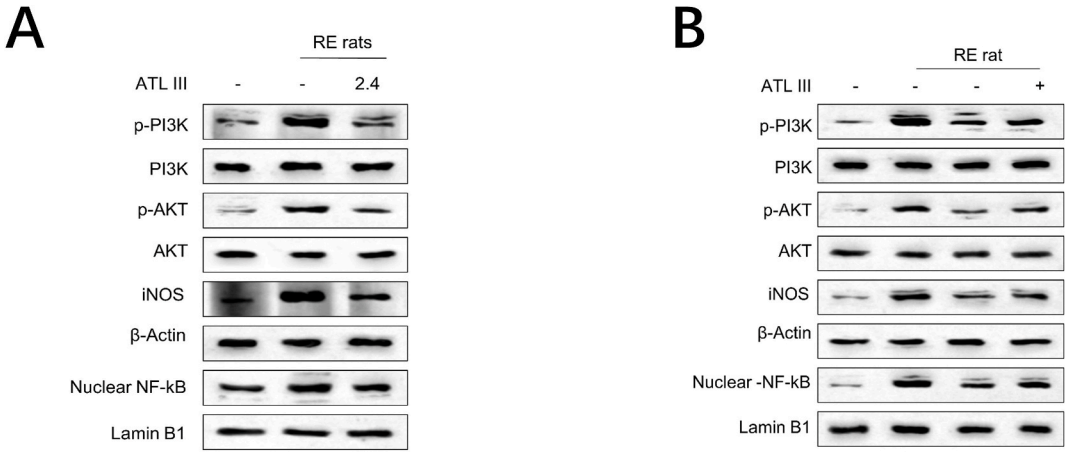


Fig. 5. A)ATL III inhibits PI3K/AKT/NF-κB in the esophageal tissues of RE rats. The Western blot analysis indicates the expression levels of various p-PI3K, *p*-AKT, iNOS, and nuclear NF-κB and Laminin B1 proteins against β-Actin as internal standard at the 2.4 mg/kg dose of ATL III. **B)** The Western blot analysis indicates the expression levels of various p-PI3K, *p*-AKT, iNOS, and nuclear NF-κB and Laminin B1 proteins against β-Actin as internal standard after treated with the ATL III +740 Y–P.

The PI3K activator 740 Y–P combined with ATL III was used to intervene in the RE rats. It was observed that the damage of esophageal tissue was aggravated, and the damage score of esophageal mucosa was increased in rats treated with the combination of ATL III +740 Y–P compared with the ATL III- alone treated group (Fig. 4A and B). In addition, the esophageal mucosal score showed a significant decrease after ATL III treatment, which showed an increase in its level after subjecting to the combination of ATL III +740 Y–P (Fig. 4C). Moreover, the ATL III +740 Y–P combination treatment showed decreased gastric content pH in RE rats compared to the ATL III- alone treated group (Fig. 4D). Consistent with the pathological detection and esophageal mucosal injury score results, the ROS and TBARS levels were significantly increased in the esophagus of rats treated with the combination of ATL III +740 Y–P compared with the ATL III alone-treated group (Fig. 4E and F). Similarly, the expression levels of various inflammatory cytokines (TNF-α, IL-8, IL-6, and IL-1β) were increased after treatment with the combination of ATL III + 740 Y–P compared with the ATL III alone-treated group (Fig. 4G).

And compared with the RE group, the expression levels of the above proteins in the esophageal tissues of rats in the ATL III (2.4 mg/kg) group were significantly decreased, indicating the action of ATL III against the notified proteins (Fig. 5A). the expression levels of p-PI3K, *p*-AKT, iNOS, and nuclear NF-κB proteins were markedly increased after being treated with the ATL III + 740 Y–P combination compared with the ATL III alone-treated group (Fig. 5B). Together, our results indicated that the amelioration effect of ATL III in the esophageal injury of the RE rats could act by inhibiting the PI3K/AKT/NF-κB/iNOS signaling pathway.

4. Discussion

Indeed, gastroesophageal reflux disease (GERD) is one of the most common gastrointestinal disorders caused by the abnormal reflux of gastric contents, possibly due to excess oil intake or abnormal diet habits [18]. RE, a common disorder in GERD, refers to esophageal inflammation with manifested symptoms of heartburn, chronic cough, pharyngeal pain, and asthma. Several therapeutic options are widely used in the clinical treatment of RE, including proton pump inhibitors and gastric motility drugs, among others. Nonetheless, these therapeutic agents suffer from a significant disadvantage of inadequate therapeutic efficacy while treating patients with RE. For instance, it is worth mentioning that 20–30 % of patients treated with the aforementioned drugs show no substantial clinical benefits, hindering their utility in addressing RE. In the current study, a rat model of RE with similar pathological features to clinical RE patients was established by hemipyloric ligation, cardia transection, and HCl perfusion. Further, it was observed that the pathological lesions in the esophageal sections of RE rats treated with ATL III were significantly alleviated. Simultaneously, the pH value of gastric tissue contents was obviously elevated, indicating their excellent dose-dependent therapeutic efficacy. The results indicated that ATL III showed a pronounced ameliorating effect on RE, providing the possibility of further investigation of ATL III as a therapeutic agent in the clinic to act against RE.

RE is a chronic disease caused by excessive reflow of the gastric contents due to the defective mechanical lower esophageal sphincter. Previous studies indicated that reflux of the gastroesophageal contents substantially enhanced the increased production of oxygen-derived free radicals that could subsequently cause damage to the esophageal mucosa [19,20]. Since oxidative stress could be a significant inducing cause of RE, studies showed that administering free radical scavengers, for instance, superoxide dismutase (SOD), almost completely suppressed the esophageal inflammatory response in rats [21]. Clinically, Wetscher and colleagues demonstrated that the production of oxygen-derived free radicals was significantly increased in the distal esophageal biopsy samples from patients with clinical RE compared to the healthy subjects. To this end, the increased production of oxygen-derived free radicals was significantly accompanied by the enhanced lipid peroxidation in the esophageal mucosa, a sensitive marker of membrane damage caused by free radicals [22]. In the current study, the experimental results indicated that ATL III could significantly reduce ROS and TBARS levels in the esophagus of RE rats, accompanied by a significant reduction in the expression levels of inflammatory cytokines (TNF-α, IL-8, IL-6, and IL-1β). The substantial inhibitory effect of ATL III on oxidative stress and inflammatory response might be a possible way to improve RE. Despite the success in exploring the actions against RE, the precise mechanism of ATL III regulating oxidative stress and inflammatory response in the pathological process of RE requires further in-depth study.

Indeed, several researchers demonstrated that intracellularly generated inflammatory factors played critical roles in RE. Activating inflammatory factors could depend on the transduction of multiple intra- and extracellular signaling pathways. In this context, some reports demonstrated that the PI3K/AKT pathway generated inflammatory responses in most living organisms, in which inhibiting PI3K/AKT could reduce the release of multiple inflammatory factors [23–26]. In an instance, Samuel and colleagues presented that the expressions of *p*-AKT and NF in injured pancreatic tissue were significantly increased during ligation of the pancreatic duct-induced acute pancreatitis in animal models, which reversed after the loosening of the ligature [27]. Notably, AKT was also reported to be associated with esophageal lesions in studies on the progression of esophageal lesions [28,29]. For instance, the inhibition of PI3K/AKT hyperactivation in a mouse model of esophageal cancer significantly contributed to the inhibition of tumor development. However, no study related to establishing the signaling pathway and RE has been reported. Considering the effect of ATL III in chronic kidney disease and neuronal injury, the condition might be ameliorated by inhibiting PI3K/AKT-mediated oxidative stress [12,14]. Thus, we hypothesized that ATL III could act this way on REs also inflicted by oxidative stress. Accordingly, in this study, we observed that ATL III could significantly inhibit the PI3K/AKT/NF-κB/iNOS signaling pathway in the esophageal tissues of RE rats, which might be a potential partial mechanism of RE improvement by ATL III. We further employed PI3K activator 740 Y–P combined with ATL III to intervene in RE rats to confirm the mechanistic view of ATL III. It was observed from the results that the therapeutic effects of ATL III on RE rats were partially abolished. Meanwhile, the inhibition of the PI3K/AKT/NF-κB/iNOS signaling pathway partially reversed the inhibitory effect. Together, the results suggested that the amelioration of RE by ATL III partially depended on the response to inhibition of the PI3K/AKT/NF-κB/iNOS signaling pathway.

5. Conclusion

In summary, ATL III showed a significant therapeutic effect on RE rats, which could be mechanistically related to the inhibition of oxidative stress and inflammatory damage mediated by implicating the PI3K/AKT/NF-κB/iNOS pathway.

Author contribution statement

Xianzhe Si; Weijie Lin: Conceived the experiments; Wrote the paper.
Zhiyao Chen: Designed the experiments; Wrote the paper.
Jie Xu: Performed the experiments; Analyzed the data.
Wenbo Huang; Feng Chen: Analyzed and interpreted the data.
Jianqing Lin: Contributed reagents, materials, analysis tools or data.
Zhijun Huang: Conceived and designed the experiments.

Funding

This study was funded by the Natural Science Foundation of Fujian Province (2021J01273), Science and Technology Plan Project of Fujian Provincial Health Commission (2021zylc48).

Declaration of competing interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

This study was supported by the Quanzhou High-level Talent Plan (2020C012R, 2022C028R).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21224.

References

[1] L. Zhang, G. Liu, X. Han, J. Liu, G.X. Li, D.W. Zou, Z.S. Li, Inhibition of p38 MAPK activation attenuates esophageal mucosal damage in a chronic model of reflux esophagitis, Neuro Gastroenterol. Motil. 27 (11) (2015) 1648–1656.
[2] K. Takeuchi, K. Nagahama, Animal model of acid-reflux esophagitis: pathogenic roles of acid/pepsin, prostaglandins, and amino acids, BioMed Res. Int. (2014), 532594.
[3] S. Darvishmoghadam, M.J. Zahedi, M.M. Hayatbakhsh Abbasi, A.A. Haghdoost, M. Khalilyzade, E. Karimi Goughari, Review of clinical spectrum of gastroesophageal reflux disease in a general population; A study from south-east Iran, Middle East J. Dig. Dis. 8 (4) (2016) 310–317.
[4] Y.J. Kim, E.H. Kim, K.B. Hahm, Oxidative stress in inflammation-based gastrointestinal tract diseases: challenges and opportunities, J. Gastroenterol. Hepatol. 27 (6) (2012) 1004–1010.
[5] Y. Erbil, U. Türkoglu, U. Barbaros, E. Balik, V. Olgac, H. Kaya, B. Cimşit, Oxidative damage in an experimentally induced gastric and gastroduodenal reflux model, Surg. Innovat. 12 (3) (2005) 219–225.
[6] M.W. Pawlik, S. Kwiecien, R. Pajdo, A. Ptak-Belowska, B. Brzozowski, G. Krzysiek-Maczka, M. Strzalka, S.J. Konturek, T. Brzozowski, Esophagoprotective activity of angiotensin-(1-7) in experimental model of acute reflux esophagitis. Evidence for the role of nitric oxide, sensory nerves, hypoxia-inducible factor-1alpha and proinflammatory cytokines, J. Physiol. Pharmacol. 65 (6) (2014) 809–822.
[7] J.S. Lee, T.Y. Oh, B.O. Ahn, H. Cho, W.B. Kim, Y.B. Kim, Y.J. Surh, H.J. Kim, K.B. Hahm, Involvement of oxidative stress in experimentally induced reflux esophagitis and Barrett's esophagus: clue for the chemoprevention of esophageal carcinoma by antioxidants, Mutat. Res. 480–481 (2001) 189–200.
[8] V.D. Corleto, S. Festa, E. Di Giulio, B. Annibale, Proton pump inhibitor therapy and potential long-term harm, Curr. Opin. Endocrinol. Diabetes Obes. 21 (1) (2014) 3–8.
[9] J.W. Kang, S.M. Lee, Protective effects of chlorogenic acid against experimental reflux esophagitis in rats, Biomol. Ther. (Seoul). 22 (5) (2014) 420–425.
[10] T.H. Kang, N.R. Han, H.M. Kim, H.J. Jeong, Blockade of IL-6 secretion pathway by the sesquiterpenoid atractylenolide III, J. Nat. Prod. 74 (2) (2011) 223–227.
[11] H. Zhao, Z.H. Ji, C. Liu, X.Y. Yu, Neuroprotection and mechanisms of atractylenolide III in preventing learning and memory impairment induced by chronic high-dose homocysteine administration in rats, Neuroscience 290 (2015) 485–491.
[12] M. Wang, R. Hu, Y. Wang, L. Liu, H. You, J. Zhang, X. Wu, T. Pei, F. Wang, L. Lu, W. Xiao, L. Wei, Atractylenolide III attenuates muscle wasting in chronic kidney disease via the oxidative stress-mediated PI3K/AKT/mTOR pathway, Oxid. Med. Cell. Longev. 18 (2019), 1875471.
[13] B. Huai, J. Ding, Atractylenolide III attenuates bleomycin-induced experimental pulmonary fibrosis and oxidative stress in rat model via Nrf2/NQO1/HO-1 pathway activation, Immunopharmacol. Immunotoxicol. 42 (5) (2020) 436–444.
[14] S. Zhu, Z. Wang, J. Yu, L. Yin, A. Zhu, Atractylenolide III alleviates isoflurane-induced injury in rat hippocampal neurons by activating the PI3K/Akt/mTOR pathway, J. Food Biochem. 45 (9) (2021), e13892.
[15] Y. Li, J.M. Wo, S. Ellis, M.B. Ray, W. Jones, R.C. Martin, A novel external esophageal perfusion model for reflux esophageal injury, Dig. Dis. Sci. 51 (3) (2006) 527–532.
[16] Jiayuan Zhuang, Effects and Mechanism of Dietary Composition of N-6/n-3 Polyunsaturated Fatty Acids on Chronic Reflux Esophagitis in Rats, Fuzhou: FJMU, 2014.
[17] M. Mihara, M. Uchiyama, Determination of malonaldehyde precursor in tissues by thiobarbituric acid test, Anal. Biochem. 86 (1) (1978) 271–278.

[18] A. Hashimoto, R. Uemura, A. Sawada, Y. Nadatani, K. Otani, S. Hosomi, Y. Nagami, F. Tanaka, N. Kamata, K. Taira, H. Yamagami, T. Tanigawa, T. Watanabe, Y. Fujiwara, Changes in clock genes expression in esophagus in rat reflux esophagitis, Dig. Dis. Sci. 64 (8) (2019) 2132–2139.
[19] X. Chen, Y.W. Ding, Gy Yang, F. Bondoc, M.J. Lee, C.S. Yang, Oxidative damage in an esophageal adenocarcinoma model with rats, Carcinogenesis 21 (2) (2000) 257–263.
[20] G.J. Wetscher, R.A. Hinder, P. Klingler, M. Gadenstätter, G. Perdikis, P.R. Hinder, Reflux esophagitis in humans is a free radical event, Dis. Esophagus 10 (1) (1997) 29–32, discussion 33.
[21] G.J. Wetscher, P.R. Hinder, D. Bagchi, G. Perdikis, E.J. Redmond, K. Glaser, T.E. Adrian, R.A. Hinder, Free radical scavengers prevent reflux esophagitis in rats, Dig. Dis. Sci. 40 (6) (1995) 1292–1296.
[22] G.J. Wetscher, R.A. Hinder, D. Bagchi, P.R. Hinder, M. Bagchi, G. Perdikis, T. McGinn, Reflux esophagitis in humans is mediated by oxygen-derived free radicals, Am. J. Surg. 170 (6) (1995) 552–556, discussion 556-557.
[23] B.L. Stiles, PI-3-K and AKT: onto the mitochondria, Adv. Drug Deliv. Rev. 61 (14) (2009) 1276–1282.
[24] M. Falasca, PI3K/Akt signalling pathway specific inhibitors: a novel strategy to sensitize cancer cells to anti-cancer drugs, Curr. Pharmaceut. Des. 16 (12) (2010) 1410–1416.
[25] J.B. Huang, Y. Ding, D.S. Huang, A.J. Liang, W.K. Zeng, Z.P. Zeng, C.Q. Qin, B. Barden, Inhibition of the PI3K/AKT pathway reduces tumor necrosis factor-alpha production in the cellular response to wear particles in vitro, Artif. Organs 37 (3) (2013) 298–307.
[26] M. Saleem, F. Afaq, V.M. Adhami, H. Mukhtar, Lupeol modulates NF-kappaB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice, Oncogene 23 (30) (2004) 5203–5214.
[27] I. Samuel, M.A. Yorek, A. Zaheer, R.A. Fisher, Bile-pancreatic juice exclusion promotes Akt/NF-kappaB activation and chemokine production in ligation-induced acute pancreatitis, J. Gastrointest. Surg. 10 (7) (2006) 950–959, 2006 Jul-Aug.
[28] S. Wang, Z. Du, J. Luo, X. Wang, H. Li, Y. Liu, Y. Zhang, J. Ma, W. Xiao, Y. Wang, X. Zhong, Inhibition of heat shock protein 90 suppresses squamous carcinogenic progression in a mouse model of esophageal cancer, J. Cancer Res. Clin. Oncol. 141 (8) (2015) 1405–1416.
[29] M. Fassan, S. Realdon, M. Pizzi, M. Balistreri, G. Battaglia, G. Zaninotto, E. Ancona, M. Rugge, Programmed cell death 4 nuclear loss and miR-21 or activated Akt overexpression in esophageal squamous cell carcinogenesis, Dis. Esophagus 25 (3) (2012) 263–268.