Screening of Glycyrrhiza Active Fractions for Prevention of Tracheal Intubation-Induced Respiratory Tract Injury (Post-Print)

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Abstract

Background: Postoperative sore throat (POST) is one of the common minor yet unpleasant postoperative complications following general anesthesia. Studies have found that licorice can effectively reduce the incidence of postoperative sore throat, but its active component remains unclear.

Objective: To screen the active fraction of licorice for preventing respiratory tract injury induced by tracheal intubation.

Methods: From April 2021 to July 2022, 60 SPF-grade Wistar rats were divided into blank group, tracheal intubation group, lidocaine group, total polysaccharide group, total saponin group, and total flavonoid group using random number table method, with 10 rats in each group. Except for the blank group, all other groups were prepared as tracheal intubation models. Before intubation, each treatment group received oral spray of 1% lidocaine, total polysaccharides, total saponins, or total flavonoids at 1 mL/100 g (based on body weight) to infiltrate the uvula and soft palate surrounding tissues, while the blank group and tracheal intubation group received oral spray of equal volume of 0.9% sodium chloride solution. After 2 hours of mechanical ventilation, the tracheal tube was removed. Under deep anesthesia, pharyngeal mucosal tissues and blood samples were collected from the rats. Pharyngeal mucosal tissues were obtained to observe pathological changes and expression levels of Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4) through hematoxylin-eosin staining (HE staining) and immunohistochemistry (IHC). Enzyme-linked immunosorbent assay (ELISA) was used to detect serum inflammatory factors [serum tumor necrosis factor-α (TNF-α), interleukin (IL)-2, IL-4, IL-10], oxidative stress [superoxide dismutase (SOD), malondialdehyde (MDA), total antioxidant capacity (T-AOC)], and stress hormones [cortisol (Cor), epinephrine (E), and norepinephrine (NE)] expression levels in rat serum.

Results: HE staining showed that the tracheal intubation group exhibited mucosal shedding, severe destruction of submucosal structures, and massive inflammatory cell infiltration, while the total saponin group showed milder destruction of pharyngeal mucosal tissues with significantly reduced inflammatory cells. ELISA results showed: compared with the blank group, serum TNF-α and IL-2 levels in the tracheal intubation group increased, while IL-4 and IL-10 levels decreased ($P<0.05$); compared with the tracheal intubation group, serum TNF-α and IL-2 levels in the total saponin group decreased, while IL-4 and IL-10 levels increased ($P<0.05$). Compared with the blank group, serum MDA level in the tracheal intubation group increased, while SOD and T-AOC levels decreased ($P<0.05$); compared with the tracheal intubation group, serum MDA levels in the total saponin group and total flavonoid group decreased, while SOD and T-AOC levels increased ($P<0.05$). Compared with the blank group, serum Cor, E, and NE levels in the tracheal intubation group increased ($P<0.05$); compared with the tracheal intubation group, serum Cor, E, and NE levels in the total saponin group decreased ($P<0.05$). IHC results showed: the average optical density (AOD) of TLR2 and TLR4 expression in pharyngeal mucosal tissues of the tracheal intubation group was higher than that in group C ($P<0.05$). Compared with the tracheal intubation group, the AOD of TLR4 expression in pharyngeal mucosal tissues of the total saponin group decreased ($P<0.05$).

Conclusion: The main active fraction of licorice for preventing respiratory tract injury induced by tracheal intubation under general anesthesia is total saponins.

Full Text

Abstract

Background: Postoperative sore throat (POST) is one of the common mild but unpleasant complications following general anesthesia. Studies have found that Glycyrrhiza Radix can effectively reduce the incidence of POST, but its effective fraction remains unclear. **Objective:** To screen the effective fraction of Glycyrrhiza Radix for preventing respiratory tract injury induced by endotracheal intubation. **Methods:** From April 2021 to July 2022, 60 SPF-grade Wistar rats were randomized into six groups (n=10 each) using a random number table: control, endotracheal intubation, lidocaine, total polysaccharides, total saponins, and total flavonoids. All groups except the control underwent endotracheal intubation model preparation. Before intubation, treatment groups received oral spray of 1% lidocaine, total polysaccharides, total saponins, or total flavonoids at 1 mL/100 g (based on body weight) to permeate the uvula and peri-soft palate tissue, while the control and intubation groups received equal volumes of 0.9% saline. After 2 hours of mechanical ventilation, the tracheal tube was removed. Under deep anesthesia, pharyngeal mucosal tissues and blood samples were collected. Pharyngeal mucosal pathology and expression levels of Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4) were observed via hematoxylin-eosin (HE) staining and immunohistochemistry (IHC). Serum in-

flammatory cytokines [tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-10], oxidative stress markers [superoxide dismutase (SOD), malondialdehyde (MDA), total antioxidant capacity (T-AOC)], and stress hormones [cortisol (Cor), epinephrine (E), and norepinephrine (NE)] were measured by enzyme-linked immunosorbent assay (ELISA). **Results:** HE staining revealed mucosal shedding and severe submucosal structural damage with massive inflammatory cell infiltration in the intubation group, while the total saponins group showed milder mucosal damage with significantly reduced inflammatory cells. ELISA results demonstrated that compared with the control group, the intubation group exhibited increased serum TNF-α and IL-2 levels and decreased IL-4 and IL-10 levels ($P<0.05$). Compared with the intubation group, the total saponins group showed decreased TNF-α and IL-2 levels and increased IL-4 and IL-10 levels ($P<0.05$). The intubation group also displayed increased MDA and decreased SOD and T-AOC levels compared with controls ($P<0.05$), whereas the total saponins and total flavonoids groups showed reduced MDA and elevated SOD and T-AOC levels ($P<0.05$). Serum Cor, E, and NE levels were higher in the intubation group than in controls ($P<0.05$), but these levels were reduced in the total saponins group ($P<0.05$). IHC results showed that the average optical density (AOD) of TLR2 and TLR4 expression in pharyngeal mucosa was higher in the intubation group than in the control group ($P<0.05$), and the total saponins group exhibited lower TLR4 AOD compared with the intubation group ($P<0.05$). **Conclusion:** Total saponins represent the main effective fraction of Glycyrrhiza Radix for preventing respiratory tract injury caused by endotracheal intubation under general anesthesia.

Keywords: Glycyrrhiza uralensis; Endotracheal intubation; Anesthesia; Ventilation, mechanical; Respiratory tract injury; Effective fraction; Total saponins

Introduction

Endotracheal intubation is the most important and safest method of respiratory support during general anesthesia. However, as an invasive procedure, it can cause various complications. Postoperative sore throat (POST) is one of the common mild but unpleasant complications after general anesthesia, with an incidence as high as 62% [1]. POST increases patient discomfort, prolongs hospital stays, and severely impacts patient satisfaction and postoperative recovery quality [2-3]. Moreover, because most cases of POST are self-limiting, they are often overlooked by clinicians, creating potential for medical disputes. With the increasing number of patients undergoing general anesthesia with endotracheal intubation and the rapid development of enhanced recovery after surgery (ERAS), prevention and treatment of POST have attracted widespread attention from anesthesiologists. A recent network meta-analysis [4] compared the efficacy of six topical agents for preventing POST from tracheal intubation and found that lidocaine was not the optimal topical agent. The study suggested selecting glycyrrhizin, corticosteroids, non-steroidal anti-inflammatory drugs, or N-methyl-D-aspartate (NMDA) receptor antagonists based on clinical

experience and patient preference to reduce POST. Currently, interventions for POST primarily focus on preoperative prevention, which provides some relief, but the incidence remains high [5]. Therefore, identifying clinically effective interventions with minimal adverse effects has become a research priority.

Glycyrrhiza Radix, derived from the dried roots and rhizomes of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat., or *Glycyrrhiza glabra* L. [6], mainly contains triterpenoid saponins, flavonoids, and polysaccharides [7], and exhibits anti-inflammatory, immunomodulatory, and antioxidant effects [8]. It is one of the most commonly used traditional Chinese medicines in clinical practice. Our previous research [9] found that Glycyrrhiza Radix spray significantly reduced tracheal mucosal edema, exudation, and inflammatory cell infiltration in rats after tracheal intubation, while decreasing serum levels of pro-inflammatory cytokines TNF-α and IL-1. Additionally, clinical studies have shown that Glycyrrhiza Radix spray can reduce the stress response to general anesthesia with tracheal intubation and the incidence of POST [10]. Evidence-based medicine also demonstrates that topical application of Glycyrrhiza Radix preparations effectively prevents post-intubation sore throat [11]. However, no studies have investigated the specific effective fractions of Glycyrrhiza Radix for preventing airway injury or sore throat after intubation. Therefore, this study established a rat model of general anesthesia with endotracheal intubation, isolated and extracted total saponins, total flavonoids, and total polysaccharides from Glycyrrhiza Radix, and examined the effects of these fractions on post-intubation respiratory mucosal pathology and serum levels of inflammatory cytokines, oxidative stress markers, and stress hormones to screen the effective fraction for preventing respiratory tract injury from tracheal intubation, providing new insights from traditional Chinese medicine for preventing such injuries.

Methods

Experimental Animals

Sixty SPF-grade Wistar rats (6-8 weeks old, half male and female, weighing 200$±$20 g) were purchased from the Experimental Animal Center of Gansu University of Chinese Medicine (certificate No. 62001000000591). All animal procedures were approved by the Animal Experiment Ethics Committee of Gansu University of Chinese Medicine (approval No. 2020-233) and conducted in accordance with the International Association for the Study of Pain guidelines for animal experiments.

Instruments and Reagents

The following instruments were used: DH-140 small animal ventilator (Shenzhen Reward Life Science and Technology Co., Ltd.), high-speed refrigerated centrifuge (5424R, German Eppendorf), microtome (RM2016, German Leica), tissue flotation dryer (KD-P, Zhengzhou Bobang Instrument Co., Ltd.), microscopic imaging system (ICX41, Ningbo Sunny Optical Co., Ltd.), and microplate

reader (ELX800, American Bio-Rad). Isoflurane was purchased from Shenzhen Reward Life Science and Technology Co., Ltd. MDA, T-AOC, and SOD assay kits were obtained from Nanjing Jiancheng Technology Co., Ltd. ELISA kits for TNF-α, IL-2, IL-4, IL-10, cortisol (Cor), epinephrine (E), and norepinephrine (NE) were purchased from Jiangsu Meimian Industrial Co., Ltd.

Preparation of Glycyrrhiza Radix Extracts

Glycyrrhiza Radix was purchased from Gansu Digital Herbal Testing Center Co., Ltd. and authenticated as the roots and stems of *Glycyrrhiza uralensis*. The herb was pulverized and passed through a 60-mesh sieve. Three portions of the powder (approximately 10.0 g each) were extracted with 200 mL water via ultrasonication for 1 hour, then centrifuged (3,000 r/min, 5 min). The supernatant was collected and precipitated with ethanol (80% ethanol, 9 hours), centrifuged again, and concentrated in a water bath to approximately 50 mL to obtain total polysaccharides (TP). For total flavonoids, the powder was extracted with 200 mL of 75% ethanol via ultrasonication for 1 hour, filtered, and the filtrate was purified using AB-8 macroporous adsorption resin, eluted with 80% ethanol, and concentrated to approximately 50 mL. For total saponins, the powder was reflux-extracted with 200 mL of 75% ethanol for 90 min, filtered, and the filtrate was purified using HPD-300 macroporous adsorption resin, eluted with 50% ethanol, and concentrated to approximately 50 mL.

Animal Grouping and Treatment

This study was conducted from April 2021 to July 2022. Sixty SPF-grade Wistar rats were randomly divided into six groups (n=10 each) using a random number table: control, endotracheal intubation, lidocaine, total polysaccharides, total saponins, and total flavonoids. All groups except the control were anesthetized with 1% pentobarbital sodium to establish the endotracheal intubation model. Before intubation, each treatment group received oral spray of 1% lidocaine, total polysaccharides, total saponins, or total flavonoids at 1 mL/100 g (based on body weight) to permeate the soft palate and uvula surrounding tissues. The control and intubation groups received equal volumes of 0.9% saline spray.

Model Preparation

The endotracheal intubation rat model was established according to reference [8]. Rats were anesthetized via intraperitoneal injection of 1% pentobarbital sodium solution (50 mg/kg). After 3-5 minutes, consciousness was lost and muscle relaxation achieved. The anesthetized rat was placed supine on a surgical board, and the board with the fixed rat was tilted approximately 45° (head-up position). The tongue was carefully pulled out of the oral cavity using surgical forceps. The operator held a laryngoscope in the left hand and a self-made tracheal catheter (16G vascular puncture needle) in the right hand, inserting the catheter into the airway during inspiration when the glottis opened. The catheter position was confirmed with a cotton swab before connecting to

the ventilator. Ventilator parameters were set at: frequency 80 breaths/min, tidal volume 5-10 mL, and inspiratory-expiratory ratio 1:1.5. Anesthesia was maintained with continuous inhalation of 1.3% isoflurane. During mechanical ventilation, the rat's condition was monitored, including chest wall movement frequency and amplitude, lip mucosa color, and any abnormal movements. If abnormalities were detected, the breathing circuit was checked promptly. After 2 hours of mechanical ventilation, isoflurane inhalation was stopped. The tracheal catheter was removed after the rat fully awakened, and the rat was returned to its cage for subsequent dissection.

Hematoxylin-Eosin (HE) Staining

Two hours after tracheal catheter removal, pharyngeal mucosal tissues were collected under deep pentobarbital sodium anesthesia (80 mg/kg) and fixed with 4% paraformaldehyde for pathological observation. Specimens were dehydrated, paraffin-embedded, sectioned at 5 m thickness, stained with HE, and observed/photographed using the Ningbo Sunny ICX41 microscopic imaging system.

Enzyme-Linked Immunosorbent Assay (ELISA)

Two hours after tracheal catheter removal, blood samples were collected from the abdominal aorta under deep pentobarbital sodium anesthesia (80 mg/kg) and centrifuged at 3,500 r/min for 10 min. Serum was collected and stored at -80°C. ELISA was performed according to kit instructions to detect inflammatory cytokines (TNF-α, IL-2, IL-4, IL-10), oxidative stress markers (MDA, T-AOC, SOD), and stress hormones (Cor, E, NE).

Immunohistochemical Detection of TLR2 and TLR4 Expression

Paraffin sections of prepared pharyngeal mucosal tissues were dewaxed with xylene, rehydrated through graded ethanol concentrations, treated with antigen retrieval solution, and incubated with 3% H_2O_2 to eliminate peroxidase activity before blocking with bovine serum. After incubation with primary and secondary antibodies, sections were washed, antigen-retrieved, developed with DAB, dehydrated, sealed, and observed/photographed using the microscopic imaging system. The average optical density (AOD) of TLR2 and TLR4 expression in each field was calculated using Image-Pro Plus 6.0 software, expressed as AOD = IOD/Area (i.e., intDen/Area).

Statistical Analysis

Data were analyzed using SPSS 22.0 software (IBM Corporation, Armonk, USA) and graphed using GraphPad Prism 9.0. Measurement data are expressed as mean ± standard deviation ($\bar{x}\pm s$). When variance homogeneity test showed no significance (α=0.05), one-way ANOVA with Tukey's post-hoc test was used for

multiple comparisons. Otherwise, Dunnett's T3 test was used for inter-group analysis. $P<0.05$ was considered statistically significant.

Results

Effects of Different Glycyrrhiza Radix Fractions on Pharyngeal Mucosal Pathology

In the control group, the mucosal and submucosal structures were intact with normal cellular morphology and no inflammatory cell infiltration. The intubation group exhibited mucosal shedding, severe submucosal structural damage, and massive inflammatory cell infiltration. Compared with the intubation group, the lidocaine group showed milder mucosal and submucosal structural damage but still had substantial inflammatory cell infiltration. The total polysaccharides and total flavonoids groups displayed relatively intact mucosal and submucosal structures with relatively reduced inflammatory cell infiltration. The total saponins group showed essentially intact mucosal and submucosal structures with near-normal cellular morphology and only scattered inflammatory cells [Figure 1: see original paper].

Effects of Different Glycyrrhiza Radix Fractions on Serum Inflammatory Cytokines

Serum levels of TNF-α, IL-2, IL-4, and IL-10 differed significantly among the six groups ($P<0.05$). Compared with the control group, the intubation group showed increased serum TNF-α and IL-2 levels and decreased IL-4 and IL-10 levels ($P<0.05$). Compared with the intubation group, the total saponins group exhibited decreased TNF-α and IL-2 levels and increased IL-4 and IL-10 levels ($P<0.05$). The lidocaine and total flavonoids groups also had lower serum TNF-α levels than the intubation group ($P<0.05$) .

Effects of Different Glycyrrhiza Radix Fractions on Oxidative Stress

Serum oxidative stress markers MDA, SOD, and T-AOC differed significantly among the six groups ($P<0.05$). The intubation group showed increased MDA and decreased SOD and T-AOC levels compared with controls ($P<0.05$). The lidocaine and total polysaccharides groups did not differ significantly from controls in these parameters ($P>0.05$). However, the total saponins and total flavonoids groups exhibited decreased MDA and increased SOD and T-AOC levels compared with the intubation group ($P<0.05$) .

Effects of Different Glycyrrhiza Radix Fractions on Stress Hormone Levels

Serum stress hormone levels (Cor, E, NE) differed significantly among the six groups ($P<0.05$). The intubation group had higher serum Cor, E, and NE levels than the control group ($P<0.05$). The lidocaine and total saponins groups

showed lower serum Cor, E, and NE levels compared with the intubation group ($P<0.05$), while the total polysaccharides and total flavonoids groups did not differ significantly from the intubation group ($P>0.05$) .

Effects of Different Glycyrrhiza Radix Fractions on TLR2 and TLR4 Expression

TLR2 and TLR4 protein expression levels in pharyngeal mucosal tissue differed significantly among the six groups ($P<0.05$). The intubation group showed higher AOD for TLR2 and TLR4 expression than the control group ($P<0.05$). The lidocaine, total polysaccharides, total saponins, and total flavonoids groups did not differ significantly from the intubation group in TLR2 expression AOD ($P>0.05$). However, the total saponins group exhibited significantly lower TLR4 expression AOD in pharyngeal tissue compared with the intubation group ($P<0.05$), while the lidocaine, total polysaccharides, and total flavonoids groups showed no significant difference [Figure 2: see original paper] .

Discussion

Endotracheal intubation is essential for maintaining airway patency in patients undergoing general anesthesia and is widely used clinically. However, the mechanical stimulation from this invasive procedure can damage the pharyngeal mucosal barrier, triggering inflammatory and stress responses that cause respiratory complications such as sore throat and cough. Although the incidence of POST can be reduced by selecting smaller tracheal tubes, using appropriate cuff pressures [12], intravenous or topical lidocaine application (including alkalinized lidocaine in the endotracheal tube cuff), and anti-inflammatory drugs such as steroids [4], these measures remain unsatisfactory. Furthermore, laryngoscope placement often induces severe sympathetic stimulation, which is particularly dangerous for patients with cardiovascular disease. Many techniques have been developed to reduce perioperative stress responses, including topical anesthesia of laryngeal mucosa, intravenous local anesthetics, short-acting opioids, or β-adrenergic antagonists [14]; however, these methods cannot effectively suppress the stress response. In recent years, traditional Chinese medicine has been widely applied in preventing and treating respiratory complications from tracheal intubation.

Modern pharmacological research indicates that Glycyrrhiza Radix has multiple pharmacological effects, including tonifying the spleen and augmenting qi, clearing heat and detoxifying, anti-inflammatory activity, antioxidant properties, and immunomodulation [15-18], making it widely applicable in respiratory diseases. Previous studies have shown that contact between tracheal tubes and tracheal mucosa in humans or pigs causes mechanical injury manifested as sore throat (in humans), tracheitis, and neutrophil activation [19-21], accompanied by increased expression of elastase, reactive oxygen species (ROS), IL-1β, TNF-α, and intercellular adhesion molecule-1 [21].

The anti-inflammatory activity of Glycyrrhiza Radix and its application in inflammatory diseases have been documented since ancient times [22]. Many scholars believe that the anti-inflammatory effects of Glycyrrhiza Radix are mediated primarily by saponins and flavonoids [23]. Triterpenoid saponins are characteristic marker compounds in Glycyrrhiza Radix, with glycyrrhizic acid and glycyrrhetinic acid as the main components [23], which exhibit similar pharmacological characteristics. Kageyama et al. [24] suggested that the anti-inflammatory effect of glycyrrhizic acid is similar to that of glucocorticoids and mineralocorticoids. Flavonoids, the second largest compound class after triterpenoid saponins in Glycyrrhiza Radix, demonstrate good anti-inflammatory effects and therapeutic efficacy in pneumonia, hepatitis, ulcerative colitis, gastritis, and other inflammatory diseases [25]. Our study found that the intubation group exhibited pharyngeal mucosal shedding, severe submucosal structural damage with massive inflammatory cell infiltration, and significantly elevated serum pro-inflammatory cytokines TNF-α and IL-2 with reduced anti-inflammatory cytokines IL-4 and IL-10. The total saponins group showed alleviated pharyngeal mucosal structural damage, reduced inflammatory cell infiltration, decreased serum TNF-α and IL-2 levels, and increased IL-4 and IL-10 levels, suggesting that the total saponins fraction has inhibitory effects on airway injury and inflammatory responses induced by tracheal intubation, consistent with findings from Liang et al. [9].

The antioxidant activity of Glycyrrhiza Radix is one of the main reasons for its widespread use. The multiple activities of triterpenoid saponin glycyrrhizic acid—including anti-radiation, neuroprotection, inhibition of mitochondrial permeability transition, prevention of ischemia-reperfusion injury, and alleviation of liver injury—are closely related to its free radical-scavenging and anti-oxidative stress effects [23]. Some scholars attribute the antioxidant activity of Glycyrrhiza Radix to its flavonoid compounds [26]. MDA is a lipid peroxidation intermediate product that indirectly reflects ROS metabolic status and the degree of oxidative tissue damage [27], serving as an important marker of oxidative stress. SOD, as an antioxidant enzyme, can reduce ROS accumulation and MDA production [28], thereby alleviating oxidative stress injury. T-AOC reflects the overall antioxidant capacity of the body [29]. Our study found that the intubation group had significantly increased serum MDA levels and decreased SOD and T-AOC levels. After Glycyrrhiza Radix extract pretreatment, the total saponins and total flavonoids groups showed reduced MDA levels and increased SOD and T-AOC levels, indicating that both total saponins and total flavonoids fractions can inhibit oxidative stress injury induced by tracheal intubation. This finding aligns with research by Wang et al. [30].

Tracheal intubation and other stressors primarily activate the sympathetic-adrenal medullary and hypothalamic-pituitary-adrenal axes, triggering a series of neuroendocrine responses that increase catecholamine and glucocorticoid secretion [31]. Therefore, plasma concentrations of Cor, E, and NE can evaluate the degree of stress response. Yang et al. [10] found through clinical research

that Glycyrrhiza Radix spray could reduce serum E and NE levels within 24 hours after tracheal extubation. Our study found that both lidocaine and total saponins groups significantly reduced serum Cor, E, and NE levels compared with the intubation group, suggesting that the stress response-inhibiting effect of Glycyrrhiza Radix during tracheal intubation may be related to total saponins.

Toll-like receptors (TLRs) are protein molecules involved in non-specific immunity [32]. Among multiple TLR subtypes, TLR4 is considered the primary mediator of sterile inflammation [33], with its main biological function being promotion of inflammatory factor synthesis and release. Studies have shown that the pro-inflammatory mediator HMGB1 binds to TLRs extracellularly, activating the NF- B pathway, inducing oxidative stress responses, and exacerbating inflammatory factor release [34], thereby mediating tissue injury. Our results showed that the intubation group had higher AOD for TLR2 and TLR4 expression than the control group, indicating that airway injury induced by tracheal intubation can activate TLR2 and TLR4 protein expression. The total saponins group exhibited significantly lower TLR4 expression AOD in pharyngeal tissue compared with the intubation group, suggesting that TLR4 plays an important role in Glycyrrhiza Radix prevention of respiratory tract injury from general anesthesia with tracheal intubation.

In summary, this study is the first to separate Glycyrrhiza Radix into total polysaccharides, total flavonoids, and total saponins fractions and examine their effects on pharyngeal tissue pathology, inflammatory cytokines, oxidative stress, stress hormones, and Toll-like receptor expression in a rat tracheal intubation model. The results demonstrate that the total saponins fraction can improve respiratory tract injury in intubated rats by alleviating inflammatory responses, reducing oxidative stress, and inhibiting TLR4 expression. Therefore, we preliminarily identify total saponins as the effective fraction of Glycyrrhiza Radix for preventing respiratory tract injury from general anesthesia with tracheal intubation.

Author Contributions

Zhang Jie and Xue Jianjun conceived and designed the experiments, supervised the project implementation, and revised the manuscript. Zhang Jie, Ding Shengshuang, Guo Min, and Xue Yang performed the experiments and serum index detection. Zhang Jie and Guo Min wrote the initial draft. Ding Shengshuang and Xu Ziqing performed HE staining and immunohistochemistry. Ding Shengshuang and Hou Huaijing conducted statistical analysis and prepared figures. All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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