

Original Research Article

Gastro-protective actions of *Aloe barbadensis* Miller mitigate ethanol-induced gastric injury in rats

Kai Zhu¹, Xia Yang¹, Chun Yang², Xiaoxue Ye², Hongxing Zhang^{3*}

¹Department of Emergency, The First Affiliated Hospital of Guangxi Medical University, ²Department of Pediatrics, ³Chinese Medical Center, Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine, Guangxi 530015, China

*For correspondence: **Email:** haomai7470@163.com; **Tel:** +86 771 218 8120

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Abstract

Purpose: To investigate the gastroprotective effect of leaf extract of *Aloe barbadensis* on ethanol-induced gastric ulcer in rats.

Methods: Healthy male Wistar rats ($n = 30$) weighing 180 - 220 g (mean weight = 200 ± 20 g) were randomly assigned to 6 groups (5 rats/group): control group, gastric ulcer group, two *Aloe barbadensis* extract groups (250 and 500 mg/kg), cimetidine group and indomethacin group. Gastric ulcer was induced via oral injection of absolute ethanol at a dose of 1 mL/kg after a 24-h fast. Gross evaluation, determination of gastric juice acidity and histological examination of gastric tissue were carried out.

Results: Treatment of gastric ulcer rats with *Aloe barbadensis* extract significantly reduced ulcerated area (UA), ulcer index (UI), and acidity of gastric juice ($p < 0.05$). Injection of 1 % carrageenan into rat hind paw led to a time-dependent increase in paw volume which peaked 3 h after injection. However, the *Aloe barbadensis* extract significantly and dose-dependently reduced the volume of inflamed paw, and inhibited edema formation ($p < 0.05$). It also markedly reduced or completely eliminated edema and leucocyte infiltration. Moreover, treatment of gastric ulcer rats with *Aloe barbadensis* leaf extract led to significant and dose-dependent reduction in gastric tissue MDA level ($p < 0.05$). Histological examination of the gastric wall showed that control rats had severe injury in gastric mucosa, accompanied by edema and leucocytes infiltration, relative to rats pretreated with extract which showed marked gastric protection and inhibition of edema and leucocytes infiltration. Moreover, the extract treatment protected the gastric surface against ulceration as indicated by reduced lesions in the treated rat model.

Conclusion: These results show that *Aloe barbadensis* mitigates ethanol-induced gastric injury in rats via inhibition of lipid peroxidation and inflammation. Thus, the extract has potentials for development into a therapeutic agent for the management of gastric ulcer.

Keywords: *Aloe barbadensis*, Gastric ulcer, Gastric mucosa, Inflammation, Lipid peroxidation

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INTRODUCTION

Gastric ulcer, a serious medical condition, predominantly affects adults in the age range of 55 to 65 years. Approximately 500,000 new

cases of gastric cancer are reported annually, with 5 million cases in the United States alone [1]. Ulcers occur when stomach acid damages the lining of the digestive tract. The major causes of gastric cancer include *Helicobacter pylori*

infection and non-steroidal anti-inflammatory drugs (NSAIDs). Upper abdominal pain is a common symptom of gastric ulcer [2]. Thirty-five percent of patients diagnosed with gastric ulcer usually suffer serious complications. Although gastric cancer-related mortality is low, the high prevalence and accompanying pain, suffering, and expense are of serious concern [3]. The pathogenesis of gastric ulcer has not been fully elucidated. Reactive oxygen species (ROS), over-secretion of gastric hydrochloric acid, mucosal hypoperfusion, excessive alcohol consumption and smoking have been linked to gastric ulcer [4]. Excessive alcohol consumption reduces bicarbonate secretion, mucus production and gastric blood flow [3]. Histamine H₂-receptor antagonists, proton pump inhibitors, antacids, and anticholinergics are generally used to treat gastric ulcer. However, some of these compounds produce serious side effects such as hematopoietic changes, hypersensitivity, arrhythmia and gynecomastia [1,5]. This has necessitated the search for novel plant-derived compounds that can effectively ameliorate the symptoms of the disease.

Traditional Chinese Medicine (TCM) has shown great promise in the treatment of gastrointestinal disorders such as gastritis and gastric ulcer [6,7]. The genus *Aloe* which belongs to the *Alliaceae* family is a succulent herb that grows to a height of 80 - 100 cm. It is an evergreen perennial native to the Arabian Peninsula, but grows wild in tropical, semi-tropical, and arid climates around the world [8]. The plant is cultivated for agricultural and medicinal purposes. *Aloe barbadensis* Miller is the most biologically active among 400 species [8]. Extracts of *A. barbadensis* Miller have been reported to exert anti-ageing, antioxidant, immunomodulatory, antibacterial, antifungal, and antiulcer effects [9-11]. The acid-reducing effect of the plant is similar to that of omeprazole. The nutraceutical properties of *A. barbadensis* Miller have been attributed to a glucomannan known as acemannan. The β -(1,4) linked polysaccharides in this plant have been shown to possess prebiotic potential, promote the growth of colonic bacteria, and probably improve gastrointestinal health [12]. This study investigated the gastroprotective effect of *Aloe barbadensis* on ethanol-induced gastric ulcer in rats.

EXPERIMENTAL

Preparation of plant extract

Fresh and healthy leaves of *Aloe barbadensis* Miller were collected from medicinal garden, Guangxi, China. The leaves were washed,

shade-dried for 8 - 12 days and pulverized using an electric blender. A portion of the powder (500 g) was exhaustively extracted with 5000 mL of distilled water (1:10, w/v) in a conical flask gently heated on a hot plate for 3 h. The extract was concentrated using a vacuum rotatory evaporator, and the resultant concentrate was freeze-dried by lyophilization.

Rats

Healthy male Wistar rats (n = 30) weighing 180 - 220 g (mean weight = 200 ± 20 g) were obtained from Laboratory of Guangxi Medical University (Guangxi, China). The rats were housed in metal cages under standard conditions: 12-h light/12-h dark cycle, average temperature of 25 °C and 48 % humidity. They were allowed free access to standard feed and clean drinking water. The study protocol was approved by the Institutional Animal Ethics Committee of Ruikang Hospital (approval no. 157/2018) Guangxi, China. The study procedures were carried out according to the guidelines of Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Experimental design

The rats were randomly assigned to 6 groups (5 rats/group): control group, gastric ulcer group, two *Aloe barbadensis* extract groups (250 and 500 mg/kg bwt); cimetidine group, and indomethacin group. Gastric ulcer was induced via oral injection of absolute ethanol at a dose of 1 mL/kg after a 24-h fast [13]. Treatment commenced 24 h after induction of gastric ulcer and lasted 14 days. Cimetidine served as standard anti-ulcer drug and was administered orally at a dose of 10 mg/kg bwt. Rats in indomethacin group were used for acute anti-inflammatory test. At the end of the treatment period, the rats were anesthetized using ketamine and xylazil, and blood was collected from their jugular veins for biochemical analysis. The rats were then euthanized and their stomachs were removed immediately.

Gross evaluation of gastric lesions

The stomach of each rat was opened along the greater curvature and washed with ice-cold normal saline. Gastric ulcer on the gastric mucosa appeared as elongated bands of hemorrhagic lesions. The length (mm) and width (mm) of each band was measured using planimeter [(10 × 10 mm = ulcer area) under dissecting microscope (× 1.8)]. The area of each ulcer lesion was measured by counting the

number of small squares (2 mm × 2 mm) covering the length and width of each hemorrhagic band [14]. The sum of the areas of all lesions for each stomach was used in the calculation of the ulcer area (UA) where the sum of small squares × 4 × 1.8 = UA mm². The ulcer index (UI) was determined as mean ulcer score [15]. Inhibition (H) was calculated as shown in Eq 1.

$$H (\%) = \{(Uac - Uat)/UA\}100 \dots\dots\dots (1)$$

where Uac and Uat are the ulcer area of control and treatment groups.

Acute anti-inflammatory test

The anti-inflammatory effect of *Aloe barbadensis* extract was determined using the carrageenan-induced rat paw edema method. Test drugs (extract and indomethacin) were administered orally for 5 days. One hour after the last treatment, edema was induced via injection of 100 μL of 1 % carrageenan in normal saline in the sub-plantar tissue of the right hind paw. Paw volume was measured using planimeter before, and 1, 2, 3, 4, and 5 h after injection of carrageenan. The results were expressed in terms of increase in paw volume calculated after subtraction of basal paw volume.

$$Edema \% = (Vt - Vo) 100 \dots\dots\dots (2)$$

where Vo = basal paw volume (mL); Vt = paw volume (mL) t after extract administration (h).

$$Inhibition\ of\ edema\ (\%) = (Ec - Et) 100 \dots\dots\dots (3)$$

Ec = edema of control group and Et = edema of treatment group.

Histological examination of gastric tissue

A portion of excised gastric tissue was fixed in 10 % formal saline and thereafter embedded in paraffin. Tissue sections (5-μm thick) were made using a microtome, stained with hematoxylin and eosin according to standard methods, and examined under light microscope. Some slides were stained with periodic acid Schiff base (PAS) to assess mucus production. ImageJ analysis software was used to determine the severity of damage to gastric mucosa [16].

Biochemical analysis

A portion of gastric tissue was homogenized in ice-cold phosphate buffered saline (PBS) containing protease inhibitor. The homogenate (20 %) was centrifuged at 15,000 rpm for 10

min at 4 °C to obtain supernatant which was used for biochemical analysis. The level of MDA was determined using commercial assay kit [17].

Statistical analysis

Measurement data are expressed as mean ± standard deviation (SD). Groups were compared using Duncan's multiple range test. All statistical analyses were performed using SPSS (21.0). Values of p < 0.05 were taken as indicative of statistically significant differences.

RESULTS

Gastroprotective effect of *Aloe barbadensis* extract

Treatment of gastric ulcer rats with *Aloe barbadensis* extract resulted in significant reduction in UA, UI, and acidity of gastric juice (p < 0.05). The effect of *Aloe barbadensis* leaf extract was similar to that of cimetidine (Figure 1). Injection of 1 % carrageenan into rat hind paw led to a time-dependent increase in paw volume that was maximal 3 h after injection. However, the extract significantly and dose-dependently reduced the volume of inflamed paw and inhibited edema formation (p < 0.05; Figure 2).

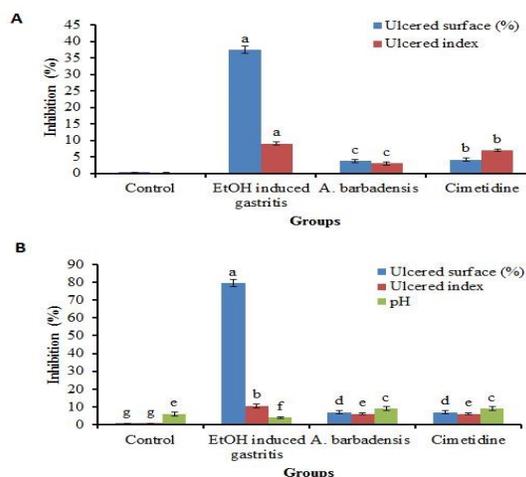


Figure 1: Effect of *Aloe barbadensis* leaf extract on UA, UI and acidity of gastric juice. (A): Ulcerated surface area (UA) and UI of rats pretreated with extract; (B): ulcerated surface area (UA), UI and pH of gastric ulcer rats treated with extract

Effect of *Aloe barbadensis* leaf extract on the histology of gastric tissue

The results of histological examination showed that the leaf extract of *Aloe barbadensis*

significantly mitigated ethanol-induced damage to the superficial layer of gastric mucosa. The gastric ulcer group showed highly extensive gastric lesions, submucosal edema and leucocyte infiltration. However, the extract treatment markedly reduced ulcer area and caused significant reduction or complete absence of edema and leucocytes infiltration. These results are shown in Figure 3.

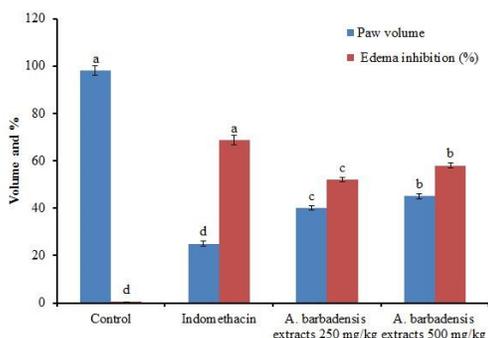


Figure 2: Effect of *Aloe barbadensis* leaf extract on paw volume and percentage inhibition of edema

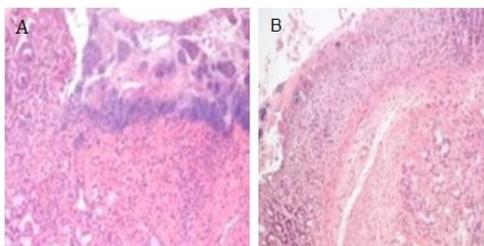


Figure 3: Effect of *Aloe barbadensis* leaf extract on the histology of gastric tissue. (A): Gastric ulceration induced by ethanol; (B): Gastric mucosa of rats treated with extract

Effect of *Aloe barbadensis* leaf extract on gastric tissue MDA level

Treatment of gastric ulcer rats with *Aloe barbadensis* leaf extract led to significant and dose-dependent reduction in gastric tissue MDA level ($p < 0.05$; Figure 4).

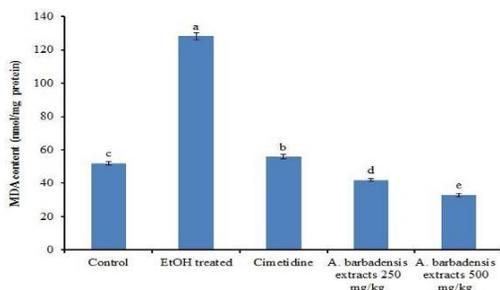


Figure 4: Effect of *Aloe barbadensis* leaf extract on gastric tissue MDA level

DISCUSSION

Gastric mucosa is the mucous membrane layer of the stomach which contains the glands and gastric pits. In humans, it is about 1 mm thick, with smooth, soft, and velvety surface. The gastric mucosa consists of simple columnar epithelium, lamina propria, and the muscularis mucosae [1]. Gastric ulcer refers to lesion or sore on the gastric mucosa resulting from gradual disintegration of surface epithelial tissue. It may be superficial, or may extend deep into underlying tissue. Gastric ulcer is one of several disorders of the upper gastrointestinal tract (GIT) caused, at least partially, by gastric acid [3]. The corrosive effects of pepsin and hydrochloric acid on the mucosa of the upper GIT contribute to gastric ulcer. Patients with gastric ulcer may present with a range of symptoms, from mild abdominal discomfort to catastrophic perforation and bleeding [2]. The pain is usually localized in the epigastrium, and it does not radiate. However, these symptoms are neither sensitive nor specific. Pain radiating to the back may suggest that the ulcer has penetrated posteriorly, or the pain may be pancreatic in origin [4]. Pain radiating to the right upper quadrant is suggestive of gallbladder or bile duct disease. Gastric ulcer pain can be best described as burning or gnawing, or as hunger pains slowly building up for 1 – 2 h, then gradually decreasing. The use of antacids may provide temporary relief. Gastric ulcer pain is often aggravated by meals [5].

The allopathic drugs used for treatment of gastric ulcer have limited clinical effectiveness. Therefore, there is a switch in attention from allopathic drugs to herbal products.

Absolute ethanol is a well-known chemical for induction of gastric injury in animals [18]. It causes gastrointestinal lesions via multifactorial pathways. Ethanol-induced gastric ulcer is a common animal model for the investigation of effectiveness of novel antiulcer drugs [19]. This study investigated the gastroprotective effect of leaf extract of *Aloe barbadensis* on ethanol-induced gastric ulcer in rats. The results obtained showed that the extract significantly ameliorated gastric injury induced by absolute ethanol. These results suggest that *Aloe barbadensis* may have cytoprotective and ulcer-healing properties, and they are in agreement with results of previous studies [4,20]. Absolute ethanol induced extensive gastric lesion, submucosal edema, acute hemorrhagic gastric erosions, sub-epithelial hemorrhages, loss of endothelial cells in blood vessels, as well as leucocytes infiltration. However, treatment with

Aloe barbadensis leaf extract resulted in marked reduction of ulcer area, flattened mucosal fold, as well as reduction or complete absence of edema and leucocyte infiltration. It is likely that *Aloe barbadensis* could effectively repair ethanol-induced gastric injury [1].

The secretion of bicarbonate into adherent layer of mucus gel creates pH gradient with a near-neutral pH at the epithelial surfaces in the stomach and duodenum, thereby providing first line of mucosal protection against luminal acid. Perturbation of the mucus-bicarbonate barrier adversely affects the mucosa and leads to loss of epithelial cells [21]. In gastric mucosa, ethanol causes gastritis via lipid peroxidation. In this study, treatment of gastric ulcer rats with *Aloe barbadensis* leaf extract led to significant and dose-dependent reduction in gastric tissue MDA level, an indication that the gastroprotective effect of the extract was exerted via suppression of lipid peroxidation in gastric mucosa. Reduced MDA level may in turn inhibit inflammation [22,23].

CONCLUSION

The results of this study show that *Aloe barbadensis* leaf extract mitigates ethanol-induced gastric injury in rats via inhibition of lipid peroxidation and inflammation.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Kai Zhu, Xia Yang, Chun Yang, Xiaoxue Ye carried out the experiments, and Hongxing Zhang has written the manuscript and designed the experiments.

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