Central Asian Journal of Medical Sciences
CAJMS

Original Article
Cent Asian J Med Sci. 2021 June;7(2):131-138. https://doi.org/10.24079/CAJMS.2021.06.008

Antioxidant Effect of GOYO-11 Against Aspirin-Induced Gastric Ulcer in Rats

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Submitted: February 16, 2021
Revised: April 14, 2021
Accepted: May 21, 2021

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Objectives: This study aimed to investigate the protective effects of GOYO-11 on aspirin-induced gastric ulcer formation. **Methods:** The prevention of aspirin-induced gastric ulcers was studied in 60 rats divided into 6 equally sized groups: healthy group (no aspirin), control group (aspirin-only), standard drug group (aspirin + omeprazole and bismuth), GOYO-11 (aspirin + GOYO-11 50 mg/kg, 100 mg/kg, 150 mg/kg, respectively). **Results:** The aspirin-only control group had distinctly attenuated activities of superoxide dismutase, catalase, glutathione peroxidase compared to the healthy group ($p < 0.05$). The control group showed a significant elevation in malondialdehyde levels compared to the healthy group ($p < 0.05$). The GOYO-11 high dose group had a significant elevation in superoxide dismutase levels compared to the control group ($p < 0.05$). The GOYO-11 middle dose group showed significant elevation in catalase, superoxide dismutase, glutathione peroxidase levels compared to the control group with a significant reduction in malondialdehyde level when compared to the control group ($p < 0.05$). **Conclusions:** GOYO-11 had a gastroprotective effect against aspirin-induced gastric ulcer rats and probably prevents ulcers by protecting the antioxidants, inhibiting lipid peroxidation (malondialdehyde), saving mucous glycoprotein, and decreasing the infiltration of inflammatory cells.

Keywords: Antioxidants, Medicine, Mongolian Traditional, Antiulcer

Introduction

Gastric ulcer disease is a prevalent disease affecting around 10-15% of the general population worldwide. Men are affected 3-4 times more than women, and 70-80% occur between the ages of 25 and 40. There are several complications in gastric ulcer disease, such as perforation 10%, bleeding10-20%, gastric outlet obstruction 10-40%, and penetration. Gastrointestinal tract diseases are the second leading cause of morbidity in Mongolia, accounted for approximately 15.5% of all morbidity over the last decade. Gastric ulcers accounted for 9.6% of diseases of the digestive system in 2019, an increase from the previous year. Generally, peptic ulcer, a common gastrointestinal pathological condition, is due to the loss of the balance between

aggressive and defensive factors of the gastric mucosa [1]. Aggressive factors that promote gastric mucosal damage include gastric hydrochloric acid (HCL) mucosal hypoperfusion [2], free oxygen radicals, and other drugs, mainly NSAIDs [3]. Aspirin, one of the most commonly used NSAIDs, is associated with gastrointestinal side effects of variable severities ranging from mild dyspepsia to severe fatal gastric bleeding. Aspirin leads to inhibition in the gastric mucosal protective factors, and at the same time, increases the aggressive factors to which the mucosa of the stomach is exposed [4].

Cynomorium songaricum, also known as "Suoyang" in China, has been widely used in traditional Chinese medicine. It is an obligate root parasitic plant and is mainly distributed in Northwestern China and Central Asia and is used as healthy foods and nutrients by local people [5]. Nowadays, the stems of *C. Songaricum* are widely used as functional food ingredients or supplements [6]. Chapter 11 of the Mongolian traditional medicine National Classification of Diseases of Basics of Theory in the Four Medical Tantra defines gastric ulcer as a chronic, persistent disease that passes through three stages: blood-yellow fever, heat and cold syndrome, and finally, the badgana-wind dominated cold stage is identified. Goyo is one of the native plants that grow in the vast Gobi Desert of Mongolia. Local people have called it the magic plant found in the Gobi Desert. There are two separate species of Goyo: the White Goyo *(Cistanche salsa)* and the Red Goyo *(Cynomorium songaricum).* White Goyo plays a vital role in maintaining the balance of the Gobi ecosystem. Due to its high treatment qualities and scarcity, collecting Goyo is prohibited, and it is registered in Mongolia's "Red Book" of protected species.

The methanol extract of *C. Songaricum* possesses potent superoxide dismutase (SOD)-like activity and moderate α-glucosidase inhibitory activity [7]. *Cynomorium songaricum Rupr* (CSR) is an essential constituent of GOYO-11, and many studies have reported on this plant's bioactivity. Many components have been isolated from *C.Songaricum* and identified. Cuiet et al. found that the significant constituents in medicinal preparations of *C.Songaricum* are flavonoids, terpenoids, steroids, organic acids, polysaccharides, saccharides, and glycosides and phloroglucinol adducts [8]. The pharmacological properties of *C.Songaricum* include improved kidney function, clearing free radicals, anti-aging, anti-oxidation, anti-fatigue, and anti-anoxia, anti-stress, anti-HIV activities, stimulating immunologic functions, enhancing sexual functions and others. Catarina Serafim et al. found that the flavonoids were able to protect the gastric and duodenal mucosa in different induction models that mimic the ulcers (ethanol, NSAIDs, stress and pyloric ligation) [9]. Multiple mechanisms of action were identified, including cytoprotective (increased mucus), antioxidative (increased activity of SOD and catalase (CAT) enzymes and glutathione peroxidase (GSH) levels, immune regulatory (reduction in pro-inflammatory cytokines and increase in anti-inflammatory cytokines), antisecretory (reduction in H^+) and anti-*H. Pylori*. Many products of natural origin, especially composed of plant foods and plants, often referred to as complementary and alternative medicines, such as nutraceuticals and herbal medicines, have stood out for their therapeutic properties, which can assist in the management of many diseases.

Unlike the studies mentioned above that primarily focus on the *C.Songaricum*, we utilized a new medication consisting of 11 ingredients, including red GOYO, based on a traditional medicine formulation used in digestive diseases.

The incidences of gastritis and gastric ulcers are increasing among the general public, along with the misuse of aspirin. There is a need for a traditional medicine with a compound effect appropriate to use without adverse side effects composed of multiple natural products. Considering the enormous potential of Mongolian medicinal flora, the aim of this study the treatment activity of GOYO used in Mongolian traditional medicine for gastric ulcers. We also aimed to tabulate the reported functions of each of GOYO's ingredients.

Materials and Methods

Drugs used

Ultrapure water 0.1g/kg; GOYO-11 50 mg/kg, 100 mg/kg, 200 mg/kg; omeprazole 4mg/kg; colloidal bismuth subcitrate (De-Nol) 700 mg/kg; and aspirin 200 mg/kg.

GOYO-11 source

Raw materials are collected and prepared according to drug-producing methods of traditional Mongolian medicine. GOYO-11 was obtained from Jing Yuan Pharmacy of China (License: Ji 20160004). Botanists defined the plants' species and family and the quality. The GOYO-11 was produced in the traditional

medical pharmaceutical factory, using proven manufacturing processes. The manufacturer performed safety tests were to check for the presence of heavy metals and toxic substances in the substrate materials. The reported function of each of GOYO-11's ingredients was obtained from *The Basic Tantra and the Explanatory Tantra from the Secret Quintessential Instructions on the Eight Branches of the Ambrosia Essence Tantra* [10].

Animals

Male Sprague Dawley rats with a bodyweight of 180 - 220 gr were used for this study. They were purchased from the National University of Inner Mongolia, Huhhot, Inner Mongolia. They were housed at 22 ± 1 ^{0}C, 65 ± 5% humidity, with a 12 h lighting cycle at the Animal Laboratory Center of Inner Mongolia Agricultural University, College of Animal Science. They were acclimatized for 1 week before beginning the experiments and were allowed free water intake and standard rodent feed. According to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and approved by the Institutional Research Committee, all the animals received good care.

Study groups

The animals were divided into 6 groups of 10 animals. The rats in the healthy group received distilled water 0.1 g/kg without any medications. Those in the control group received aspirin 200 mg/kg alone. The animals in the standard drug therapy group (omeprazole + bismuth) were given aspirin 200 mg/kg, colloidal bismuth subcitrate 700 mg/kg, and omeprazole 4 mg/kg. Three doses of GOYO-11 were studied. The low dose group received aspirin 200 mg/kg and GOYO-11 50 mg/kg. The middle dose GOYO-11 group rats received aspirin 200 mg/kg and GOYO-11 100 mg/kg. The high dose GOYO-11 group received aspirin 200 mg/kg and GOYO-11 150 mg/kg. All treatments were given orally daily in the morning for 28 days for all groups. The aspirin and treatment drugs were given concurrently.

Experimental sequence

After 28 days of treatment, the animals were sacrificed by cervical dislocation, their blood was drawn, and their stomach was removed. The tissues were then washed with ice-cold saline and examined macroscopically for mucosal lesions. After the photographs were taken, the freshly excised stomach was divided into two parts. One was processed for histological specimens and glycoprotein determination. The other part was stored at −80 C pending biochemical analyses.

Acute and chronic toxicity of GOYO-11 drugs

The acute toxicity was determined according to the five toxicological categories of substances by Sidorov after intragastric administration of GOYO-11 suspension to mice [11], and the active dose was determined by using Zapadnyuk's technique [12].

Measurement of serum glutathione peroxidase enzyme activity (GSH-Px)

Serum GSH-Px activity was measured using spectrophotometry (Nanjing Jiancheng Biotechnology Institute, China). The GSH-Px activity was determined by measuring the rate of NADPH oxidation at 340 nm using H_2O_2 as the substrate.

Measurement of serum superoxide dismutase enzyme activity (SOD)

Serum SOD activity was measured using spectrophotometry (Nanjing Jiancheng Biotechnology Institute, China). This assay depends on the ability of the SOD to inhibit the phenazinemethosulphate-mediated reduction of nitrobluetetrazolium dye.

Measurement of serum catalase enzyme activity (CAT)

Serum CAT activity was measured using spectrophotometry (Nanjing Jiancheng Biotechnology Institute, China). H_2O_2 reacts with CAT. The test is based on the reaction of H_2O_2 with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone, producing a colored chromophore that was measured at 510 nm.

Measurement of serum lipid peroxide measured as malondialdehyde (MDA)

Serum MDA was measured using spectrophotometry (Nanjing Jiancheng Biotechnology Institute, China). The color formed was measured at an optical density of 535 nm.

Photographic gastric mucosal lesion assessment

The internal surface of the stomach was photographed with a

CANON camera (EOS 80D), and the images were analyzed with Image J software (Wayne Rasband, NIH) to measure the total lesion area [13].

Histopathological examination of gastric mucosa

The stomach tissue specimens were fixed in 10% formalin, dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. The tissues were then cut into 3–5 μm thick sections by a microtome, fixed on the slides, stained with hematoxylin-eosin (H&E) and observed for pathological changes using ordinary light microscopy.

Statistical analysis

The original data were not normally distributed, so a log transformation was performed, and we used the log-transformed data for our analyses. The mean values of continuous variables were compared using one-way ANOVA. The multiple comparisons between the groups were carried out using the Tukey test. A critical p-value of < 0.05 was used. SPSS version 24 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Ethical statements

The experimental protocols were designed under ethical guidelines, and the study was approved by the Research Ethics Committee of Mongolian National University of Medical Sciences (No 2017/3-05). *Cynomorium songaricum* is a naturally abundant species in Mongolia with no restrictions on consumer usage.

Results

We studied GOYO-11, which is mainly formulated by *Cynomorium songaricum* according to the traditional pharmaceutical theory and techniques in the treatment of gastric ulcers. GOYO-11 includes 11 herbs (Table 1).

Regarding GOYO -11's acute toxicity, the LD50 was 35.1 (31.1 - 37.1) gr/kg. The active dose, ED, was 720 (360-1440) mg/kg. The aspirin-only control group had distinctly attenuated activities of CAT, SOD, GSH-Px compared with the healthy group ($p < 0.05$). The control group showed significant elevation in MDA levels when compared to the healthy group (21.72 ± 1.1 vs. 11.95 ± 1.5, $p < 0.05$) (Table 2).

Effect of high dose GOYO-11 on tissue oxidative stress parameters (MDA, CAT, SOD and GSH-Px)

The GOYO-11 high dose group showed significant elevation in SOD levels when compared to the control group 165.45 ± 19.1 vs. 116.11 ± 10.12 units, $p < 0.05$) (Table 2).

Effect of GOYO-11 middle dose on tissue oxidative stress parameters (MDA, CAT, SOD and GSH-Px)

Compared to aspirin-only control group, the GOYO-11 middle dose group showed significant elevations in the levels of CAT (13.66 ± 1.2 vs. 15.98 ± 0.56 vs, $p < 0.05$), SOD (13.66 ± 1.2 vs. 15.98 ± 0.56, $p < 0.05$), and GSH-Px (33.44 ± 9 vs. 37.73 ± 1.5, $p < 0.05$) (Table 2).

Table 1. Ingredients of GOYO-11 and their functions.

Mongolian nomenclature	Latin nomenclature	Dose (gr)	Function
Red GOYO	*Cynomorium songaricum*	20	Improves digestion and strengthens the body
Biblin	*Piper longum L.*	10	Aids food absorption and digestion
Jonsh	*Calcitum*	10	Gastroprotective effect, prevents NSAID complications and reduces toxicity
Anar	*Punicagranatum L.*	10	Aids food absorption and digestion
Gurgem	*Carthamustinctorius L.*	10	Antipyretic
Jurur	*Gardenia jasminoides*	10	Antipyretic
Sugmel	*Elletariacardamomum*	10	Supports the function of the digestive system
Ruda	*Saussurealappa*	10	Analgesic
Bashaga	*Dianthus superbus L*	10	Antipyretic
Mana	*Inulahelenium L.*	10	Aids food absorption and digestion
White GOYO	*Areca catechu*	15	Aids food absorption and digestion

Table 2. Effect of GOYO-11 on serum MDA, CAT, SOD, GSH-Px activities. All groups except the healthy group received aspirin to induce ulceration.

	Groups						
	Healthy (n=10)	Control (n=10)	Standard drugs (n=10)	GOYO-11 (50mg/kg) (n=10)	GOYO-11 (100mg/kg) (n=10)	GOYO-11 (200mg/kg) (n=10)	p-value
CAT (pg/ml)	16.86 ± 0.45	13.66 ± 1.2	14.54 ± 1.09	15.51 ± 2.21	15.98 ± 0.56	15.63 ± 0.49	0.632
SOD (pg/ml) [a, b, c, d]	132.55 ± 16.8	116.11 ± 10.12	127.78 ± 2.18	131.87 ± 11.2	149.99 ± 9.1	165.45 ± 19.1	0.000
MDA (nmol/l)	11.95 ± 1.5	21.72 ± 1.1	11.23 ± 1.97	12.45 ± 1.9	13.33 ± 3.1	13.89 ± 0.98	0.295
GSH-Px (pg/ml)	36.88 ± 7.8	33.44 ± 9.1	34.23 ± 1.78	35.55 ± 6.6	37.73 ± 1.5	37.02 ± 3.09	0.533

CAT- catalase, SOD- superoxide dismutase, MDA- malondialdehyde, GSH-Px- glutathione peroxidase. Control-Aspirin 200 mg/kg, Standard drugs-bismuth 700mg/kg + omeprazole 4 mg/kg. [a]Healthy vs control, p = 0.031; [b]Control vs standard drugs, p = 0.046; [c]Control vs GOYO-11(50mg/kg), p = 0.001; [d]Control vs GOYO-11(100 mg/kg), p = 0.021.

Effect of GOYO-11 on the severity of gastric lesions

No disturbance of gastric mucosa was seen in the healthy group (Figure 1a). Aspirin produced extensive visible hemorrhagic necrosis in the gastric mucosa (Figure 1b). Injuries to the gastric mucosa were much milder compared with the injuries seen in the ulcer control rats (Figure 1c).

Moderate injuries to the gastric mucosa were seen in rats treated with GOYO-11 – 50 mg (Figure 1d). Mild injuries were observed in the gastric mucosa, and flattening of the gastric mucosa was seen GOYO-11 100 mg (Figure 1e). Nearly normal gastric mucosa tissues in those treated with GOYO-11 200 mg (Figure 1f). The total lesion area and ulcer percentage decreased with increasing GOYO-11 dose. At the dose of 100 mg/kg (middle dose group), the beneficial effects of GOYO-11 were seen. The rats in the GOYO-11 middle dose group showed a significant reduction in total lesion area (376763 ± 23 vs. 12776 ± 34 mm^2, $p < 0.05$) and ulcer percentage when compared to the aspirin-only control group (5.59 ± 0.4 vs. 1.14 ± 0.08 percent, $p < 0.05$).

GOYO-11 on gastric mucosal histological changes

The control rats' stomach histology sections were structurally normal, having the normal epithelial architecture and laminal propria, submucosa and muscularis propria (Figure 2a).

In contrast, treatment with aspirin alone resulted in severe epithelial erosion, necrotic and distorted glands accompanied by

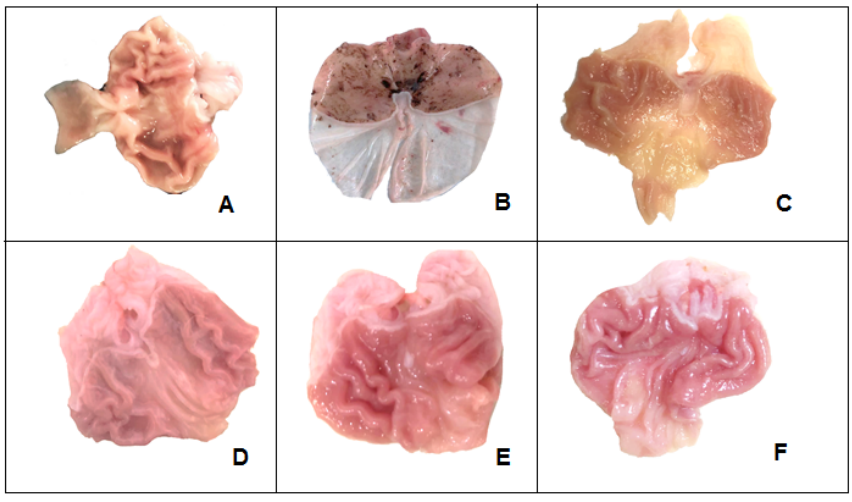

Figure 1. Representative examples of stomach lesions: (A) healthy group, (B) control group (aspirin-only), (C) omeprazole+ bismuth group, (D) GOYO-11 low dose (50 mg/kg), (E) GOYO-11 middle dose (100 mg/kg), and (F) GOYO-11 high dose (200 mg/kg). All groups except the healthy group received aspirin and underwent pyloric ligation to induce ulceration.

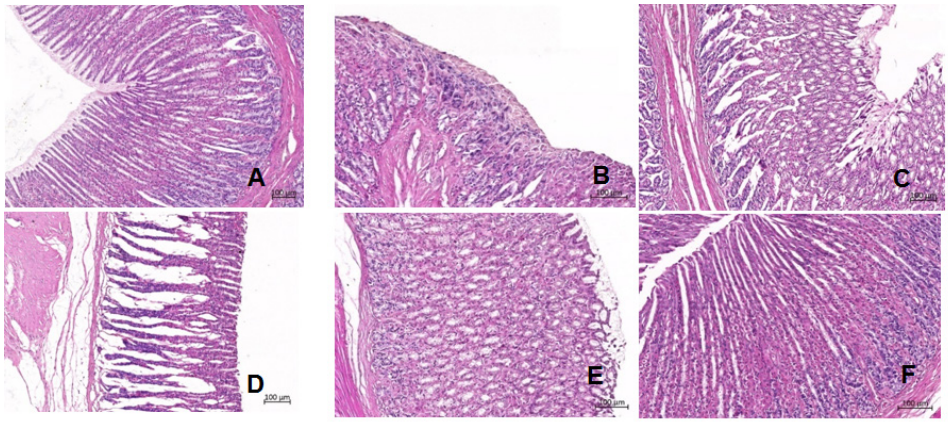

Figure 2. Representative examples of stomach stained with H&E at 400x magnification. (A) healthy group, (B) control group (aspirin-only), (C) omeprazole+bismuth group, (D) GOYO-11 low dose (50 mg/kg), (E) GOYO-11 middle dose (100 mg/kg), and (F) GOYO-11 high dose (200 mg/kg). All groups except the healthy group received aspirin and underwent pyloric ligation to induce ulceration.

marked cellular infiltration by mononuclear cells, degenerative changes in forestomach and fundic regions (Figure 2b). Examination of gastric mucosa in the standard drug group and GOYO-11 low dose group showed shedding of the mucosa forming sharply demarcated ulcers with hemorrhage and infiltration of acute inflammatory cells, primarily neutrophils, in all layers of the gastric wall (Figure 2c). Conversely, microscopy of the GOYO-11 middle dose group showed a mild degree of inflammation (Figure 2d) and infiltration by acute inflammatory cells, mainly neutrophils and macrophages, compared with the GOYO-11 low dose group (Figure 2e). In addition, the tissues of the GOYO-11 high dose group appeared nearly normal compared to the aspirin-only control group with insignificant minimal inflammatory infiltration (Figure 2f).

Discussion

Non-steroidal anti-inflammatory drugs induced gastric mucosal damage is one of the important causes of morbidity globally (14). NSAIDs are the second most common cause of gastric ulcers, particularly in seniors [15]. With age, the capacity of the gastric lining to resist damage decreases which in turn increases the risk of gastric ulcer disease, especially in people who use aspirin and other NSAIDs [16]. Aspirin was the first discovered member of the class of drugs known as NSAIDs, not all of which are salicylates. However, they all have similar effects, and most have some mechanism of action that involves non-selective inhibition of the enzyme cyclooxygenase. Although the mechanisms of NSAID-induced gastric injury are not well understood, it is widely accepted that both COX-dependent and -independent mechanisms are involved. NSAIDs, ethanol, and acetic acid induce gastric damage using different mechanisms; they all result in the formation of oxidative stress and a decrease in protective mucosal factors [17]. Furthermore, various factors such as the stimulation of gastric acid production, inflammatory cell infiltration, cytokines, mucosal blood flow, and free radicals are known to contribute to the development of NSAID-induced gastric mucosal damage [18].

We decided to use SD rat in our present study, which was the commonly used rat strain in the previously published papers regarding NSAID-induced enteropathy [19]. *Cynomorium songaricum* has been used in Chinese traditional medicine, and it was reported to exert some bioactivities, such as scavenging hydroxyl radicals and superoxide radicals. Furthermore, we showed a protective effect of GOYO-11 against oxidative stress in vivo using an aspirin-induced gastric ulcer rat model. We found the sharp and pungent effects were lower than we expected.

In this study, as shown in Figure 1, we demonstrated that GOYO-11 not only attenuated the inflammation response in the gastric mucosa but also decreased both the number and the size of gastric mucosal lesions as well as decreased mucosal defects, erosion, hyperemia. The findings demonstrated that GOYO-

11 has a tremendous potential value in treating acute gastric mucosal gastric lesions caused by NSAIDs. When we measured the area of NSAID-induced damage, there was a statistically significant difference between GOYO-11 low doses treated, GOYO-11 middle dose and high dose group. Both the gross ulcer index and the damaged area increased in a dose-dependent manner by GOYO-11. Aspirin-induced gastric ulcer causes an inflammatory response associated with increased neutrophil infiltration disturbing the oxidant/antioxidant balance. With age, the capacity of the gastric lining to resist damage decreases which in turn increases the risk of gastric ulcer disease, especially in people who use aspirin and other NSAIDs. Omeprazole was used as a positive control in this study because it is widely used for treating gastric ulcers and has been shown in numerous published studies to provide a gastroprotective effect [20-22].

To our knowledge, this is the first animal study to evaluate the protective effects of GOYO-11 against aspirin-induced gastric ulcers. Based on the fundamental theories of the Traditional Mongolian Medicine, the therapeutic effects of GOYO-11 formulation, which is mainly formulated by *Cynomorium songaricum* according to the traditional pharmaceutical theory and techniques, has been studied in the process of the treatment of gastric ulcer. Cuiet et al. [8] found that the *Cynomorium songaricum* protected the gastric mucosa against different ulcer-mimicking induction models (ethanol, NSAIDs, stress and pyloric ligation) through multiple mechanisms of action, such as cytoprotection, antioxidants. The present study demonstrated the gastroprotective effect of three different doses of GOYO-11 on aspirin-induced gastric ulcers in rats. Aspirin produced obvious macroscopic stomach ulcers compared with the healthy group. It also increased the percentage of neutrophils in the gastric mucosa and increased MDA levels compared with the healthy group. The GOYO-11 50, 100, and 200 mg/kg groups, similar to omeprazole and bismuth, significantly decreased the percentage of neutrophils in the gastric mucosa and increased gastric secretion.

This study showed that GOYO-11 at a dose of 100 mg/kg has a protective effect on the development of gastric ulcers induced by aspirin, possibly by inhibiting the MDA enzyme and increasing activities of SOD level in the serum. Future experiments are needed to investigate the protective effects of the active ingredients of GOYO-11 in reducing gastrointestinal damage and healing gastric ulcers.

Conclusion

GOYO-11 prevents aspirin-induced gastric ulcers and probably does this by protecting the antioxidants, inhibiting lipid peroxidation (MDA), saving mucous glycoprotein, and decreasing the infiltration of inflammatory cells.

Conflict of Interests

The authors declare they have no conflict of interest regarding the publication of this paper.

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