

Experimental Investigation on the Therapeutic Potential of Kaempferol-4-O-β-D-glucopyranoside from Citrullus colocynthis in the Management of Functional Dyspepsia

Hemalatha^{1*}, Swarnalatha², Karthi³

^{1,2} Department of Pharmacology, Pallavan Pharmacy College, Kanchipuram, Tamil Nadu, India

³ Department of Pharmacognosy, Pallavan Pharmacy College, Kanchipuram, Tamil Nadu, India

ABSTRACT

Functional dyspepsia is a prevalent gastrointestinal disorder characterized by impaired gastric motility, secretion, and mucosal inflammation. The present study evaluates the therapeutic potential of Kaempferol-4-O-β-D-glucopyranoside (K-4-O-β-D-g), isolated from Citrullus colocynthis fruit, against functional dyspepsia (FD) using a loperamide-induced mouse model. K-4-O-β-D-g was extracted, isolated, and assessed for safety via acute oral toxicity testing, revealing moderate toxicity at high doses (LD₅₀ = 1,311.45 mg/kg). Mice treated with K-4-O-β-D-g (50 and 100 mg/kg) showed significant improvements in gastric emptying, intestinal transit, and gastric smooth muscle contractility, comparable to the reference prokinetic agent mosapride. Moreover, K-4-O-β-D-g enhanced pepsin activity and secretion, mitigated ethanol-induced gastric mucosal damage, and reduced inflammation and necrosis as confirmed by histopathological analysis. Molecular investigations revealed upregulation of neuronal nitric oxide synthase (nNOS) and motility-associated genes (5-HT₄R, RYR3, ANO1, smMLCK), along with increased serum serotonin levels, underlying the compound's multifaceted mechanism of action in restoring gastrointestinal function. These results substantiate the traditional use of Citrullus colocynthis and highlight K-4-O-β-D-g as a promising multitarget candidate for FD treatment, warranting further chronic and clinical investigations.

Keywords: Functional dyspepsia, Kaempferol-4-O-β-D-glucopyranoside, Gastric emptying, Intestinal transit, Gastric smooth muscle contractility.

INTRODUCTION

Gastrointestinal disorders, or GITs, encompass a range of conditions primarily affecting the GI tract, including Gastroesophageal reflux disease (GERD), Inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and colorectal cancer (CRC), among others. The prevalence of major gastrointestinal conditions is considerable worldwide. Functional dyspepsia is a particularly common disorder, impacting more than 20% of the population (Thompson, 2017). This clinical syndrome features chronic symptoms originating from the gastroduodenal area and is divided into three subcategories: epigastric pain syndrome, postprandial distress syndrome (PDS), and a combination of both, each thought to arise from different underlying mechanisms (Francis et al, 2024). The underlying

mechanisms of functional dyspepsia are intricate, involving numerous factors at both macroscopic and microscopic levels. Macroscopically, issues may include delayed or rapid gastric emptying, gastric dysrhythmias, antral hypomotility, and visceral hypersensitivity, with altered nervous system processing and dysfunction in mechano-receptors. Microscopically, the condition is characterized by impaired mucosal barrier function, sensitivity to duodenal acids or lipids, and resulting gastritis, which involves increased eosinophils, mast cells, and gut-specific lymphocytes linked to cytokine release, such as TNF-α. Furthermore, the condition can be exacerbated by altered gut microbiota and Helicobacter pylori infections. Psychological factors, such as anxiety, depression, and stress, influence central nervous system processing by hyperactivating the amygdala and disrupting the hypothalamic-

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

pituitary-adrenal (HPA) axis, leading to increased cortisol levels. Managing dyspepsia requires a thorough evaluation, including patient history, physical exams, and checking for alarm symptoms like weight loss, vomiting, dysphagia, bleeding, and family history of GI cancer. Initial management typically involves dietary changes, such as consuming smaller meals and avoiding high-fat foods, alongside testing and treating *H. pylori* infections, which can improve symptoms over time. Medications such as PPIs or H2 receptor antagonists are commonly used for acid suppression, usually for about eight weeks, and evidence supports their effectiveness. In cases that do not respond to other treatments, tricyclic antidepressants may be beneficial, especially for those with psychiatric comorbidities (Yamawaki et al, 2017). Current treatments for functional dyspepsia (FD) face various limitations and challenges. Although some medications provide partial symptom relief, they only reduce symptoms effectively in around one-sixth of patients. This limited success has led to the exploration of alternative remedies beyond mainstream Western medicine. A significant challenge is the incomplete understanding of FD's pathophysiology, which complicates the development of targeted treatments. Moreover, the overlapping symptoms of FD with conditions like IBS and GERD can make diagnosis and treatment more difficult. There are currently no medications specifically approved for FD, resulting in the use of off-label treatments. Some therapies may also have side effects that restrict their long-term use, and the variability of FD's symptoms adds to the complexity of effective management.

Traditional medicine includes the “knowledge, skills, and practices that are based on the theories, beliefs, and experiences specific to different cultures, used for maintaining health and for the prevention, diagnosis, enhancement, or treatment of both physical and mental health issues.” The main reasons for choosing traditional medicine involve its cost-effectiveness, its compatibility with patients' beliefs, the alleviation of concerns regarding the side effects of chemical (synthetic) drugs, the desire for more personalized healthcare, and improved access to health information for the public. (Benzie et al 2011). With technological advancements, it has become feasible to identify various components in herbal extracts that hold

medicinal value and optimize their use. Furthermore, discovering the active compounds in herbal extracts has allowed researchers to investigate their mechanisms, conduct clinical trials, and make chemical alterations to create more effective molecules. Recently, there has been a notable increase in global interest in herbal medicines for functional gastrointestinal disorders (FGIDs), as shown by the rising volume of publications concerning herbal treatments. (Gwee et al 2021)

Citrullus colocynthis is a significant medicinal plant in the cucurbit family, Cucurbitaceae. The medicinal properties of *C. colocynthis* are very important for traditional medicine practices. The pharmacological benefits of its roots, fruits, seeds, leaves, and the entire plant have been utilized in treating various diseases. (Sharma et al, 2022). This study focuses on the fruits of *Citrullus colocynthis*, which are traditionally used for addressing gastrointestinal ailments.

MATERIALS AND METHODS

MATERIALS

Fresh fruits of *Citrullus colocynthis* was collected from the local vendors of Kanchipuram district of Tamilnadu. It was authenticated by Botanist Dr. K N Sunil Kumar of Siddha Central Research Institute, Arumbakkam, Chennai (Code No:C09092505C)

The following reagents used were domestic and of analytical grade 1,1,2-trichloroethane, acetylcholine, chloroform, ethyl acetate, Evans blue, formalin, hydrochloric acid, methanol, n-hexane, paraformaldehyde, phenol red, polyacrylamide gel, sodium hydroxide, trichloroacetic acid, loperamide, mosapride.

EXTRACTION AND ISOLATION OF CITRULLUS COLOCYNTHIS FRUIT

The collected fruits of *Citrullus colocynthis* were cleaned, chopped into small pieces, air-dried in shade, and mechanically ground into fine powder filtered through a 60# mesh. Fifty grams of the powder were extracted using a Soxhlet apparatus with 70% methanol (1:5 w/v) for 72 hours at 37°C. The methanolic extract was filtered and concentrated under reduced pressure using a rotary evaporator.



The concentrated extract was suspended in chloroform and subjected to sequential liquid-liquid extraction with chloroform and ethyl acetate, yielding 10.4 g and 48.7 g of respective fractions. The chloroform fraction showed no specific TLC spots and was excluded from further study. The ethyl acetate fraction (EAF) was chromatographed by flash column on silica gel eluted with a chloroform-methanol gradient (100:0 to 4:1). Based on TLC profiles, collected fractions were combined into seven groups.

Fraction 4 was further purified by Sephadex LH-20 column chromatography using chloroform-methanol (1:1) at 1 mL/min and 30°C, monitored at 260 and 370 nm UV wavelengths, yielding compounds Quercetin (0.65 mg) and Kaempferol (0.52 mg) for spectral analysis. Fraction 7 was purified on Sephadex LH-20 with methanol then on ODS RP-C18 column with methanol-water (40-60%) gradient monitored at 254 nm, yielding compound Kaempferol-4-O- β -D-glucopyranoside (1.80 g) which was recrystallized for further pharmacological study.

Due to low yield, compounds Quercetin and Kaempferol were only characterized spectrally, while compound Kaempferol-4-O- β -D-glucopyranoside was selected as the main test compound for anti-dyspeptic activity evaluation.

ANIMALS AND EXPERIMENTAL DESIGN

Balb/C male mice ranging from 25-30 g and were five weeks old at the time of purchase from Mass biotech Lab Reasearch, Chengalpattu. The mice were housed in a temperature-controlled room within a specific pathogen-free (SPF) facility, where the temperature were maintained at 23 ± 2 °C and the relative humidity at $60 \pm 5\%$. The mice followed a 12/12 h light/dark cycle in their housing environment. The mice were provided with unlimited access to commercial standard diet and water. Following a one-week acclimatization period, the mice were randomly divided into five groups (6 mice/ group). Animals in group I, which served as control group, received distilled water, animals in group II, which served as negative control group, received intraperitoneal administration of lopermide (10mg/kg), animals in group III & IV, which served as treatment groups, received oral administration of two different doses

of K-4-O- β -D-g (compound 3) (50, and 100 mg/kg b.w.), and animals in group V, which served as positive control group, received mosapride (3 mg/kg). All experimental procedures were carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and supervision on Experimentation on Animals and were approved by the Institutional Animal Ethics Committee (IAEC NO:2318/PO/Re/S/2025/CPCSEA) from Pallavan pharmacy college, Kanchipuram.

ACUTE ORAL TOXICITY (AOT) TEST

The acute oral toxicity (AOT) study for compound 3 was conducted using Balb/C male mice. The conditions for the study were set according to the OECD 423 guideline.

The mice were classified into four groups, each containing three mice. They were allowed unlimited access to a standard commercial diet and water. Each mouse was marked for identification using a 1% solution of picric acid.

All animals were administered Kaempferol-4-O- β -D-glucopyranoside (K-4-O- β -D-g) at the following doses: 500, 1000, 1800, and 2000 mg/kg, respectively. The control group was administered an injection of saline solution. Following the administration of K-4-O- β -D-g, each animal was monitored individually every hour on the first day and daily for the following 14 days.

Behavioral changes and clinical signs in the animals were recorded throughout the experiment. Body weights were measured prior to dosing and at designated intervals during the observation phase to assess any treatment-related effects, as well as morbidity and mortality over the 14-day period. The dosage for this study has been determined based on the findings from this research.

BODY WEIGHT, FOOD, WATER CONSUMPTION, AND BEHAVIORAL CHANGES

The behavior of mice were observed every day, including lazy movement, squint, hair color, weight, and defecation frequency; the mice were fed a weighed solid diet. At 8 a.m. on the next day, the



remaining amount of the diet were measured. The growth rate of body weight were calculated, the 3-h food intake (overnight fasting mice were allowed free access to food and water for 3 h) and 24-h food intake were recorded.

GASTRIC WEIGHT AND GASTRIC EMPTYING USING PHENOL RED

The mice underwent a 19 h fasting period with unrestricted access to distilled water. The choice of phenol red solution volume (500 μ L/mouse) and the time point of 50% delayed gastric emptying (30 min after intraperitoneal injection with loperamide (10 mg/kg) were made based on previously established study protocols. After a 30 min period following the administration of 0.05% phenol red (dyeing substance that checks the level of gastric emptying), the mice were humanely euthanized. The stomachs were promptly excised and weighed. Subsequently, the stomachs were treated with 5 mL of 0.1 N sodium hydroxide solution to measure the optical density of residual phenol red. Additionally, 0.5 mL of trichloroacetic acid (20% w/v) were added to the stomachs. The produced homogenate were centrifuged at 3000 rpm for 20 min and then mixed with 0.5 N sodium hydroxide solution with supernatant 1 milliliter. In addition, the optical density were measured at a wavelength of 560 nm with a spectrophotometer.

The emission values stated earlier were calculated using the formula provided below:

$$\text{gastric emptying (\%)} = (1 - X/Y) \times 100$$

X: The optical density of the phenol red that remains on the surface. Y: The optical density of the phenol red mixed with sodium hydroxide measured under test tube conditions.

INTESTINAL TRANSIT RATE BY EVANS BLUE

To measure the intestinal transit rate, the Evans blue diet method were used, in which 5% Evans blue (dyeing substance that checks the level of intestinal transit rate) were prepared in distilled water. Evans blue diet were orally administered (250 μ L/20 g mouse) 30 min after IP injection of loperamide

(10mg/kg). After a 30-minute interval post the administration of Evans blue dye, the mice were compassionately euthanized. The progression of the Evans blue dye within the small intestine, particularly from the pylorus to the cecum, was measured to assess the distance of intestinal transit.

GASTRIC SMOOTH MUSCLE ACTIVITY RECORDING

An ex vivo analysis of isometric contractions of the longitudinal muscle strips assessed the function of the gastric smooth muscle. In brief, after the mucosal layer was removed, the muscle strips (10 mm long and 3 mm wide) were prepared along the longitudinal direction of the tract. Subsequently, these strips were placed in individual 10-ml chambers filled with oxygenated Krebs' solution (95% O₂ + 5% CO₂) containing 119 mM NaCl, 4.7 mM KCl, 1.2 mM NaH₂PO₄, 25 mM NaHCO₃, 2.5 mM CaCl₂, 1.2 mM MgSO₄, and 11.1 mM glucose, maintaining a pH of 7.30–7.40, while being held at a stable temperature of 37°C on an organ bath apparatus. One end of each strip was secured with thin wires, with the opposite end linked to a TRI201AD Isometric Transducer, which was connected to a Quad Bridge Signal Amplifier and a PowerLab 8/35 analog-digital converter. A preload of 1.5 g was applied to every strip, and the contractile curves were recorded and analyzed using LabChart software version 7.0. The strips underwent a 30-minute equilibration period to establish stable spontaneous contractions. The spontaneous activity of the smooth muscle was documented, and the frequency, amplitude, and motility index (MI) of the spontaneous contractions were computed. The MI is defined as the area under the contractile curve (AUC) per unit time, representing the overall contractility of smooth muscle strips, which includes tension, amplitude, and frequency. Various doses of ACh (1, 2, 4, 6, 8, 16, 32, 64 μ g) from the stock solution (10 μ g/ml) were administered into the tissue bath until the maximum ceiling effect was achieved. The contractile curves were recorded, and the half-effective concentration (EC₅₀) for ACh was determined.

DETERMINATION OF DIGESTIVE ENZYMES

Each group of mice underwent a 24-hour fasting period. Subsequently, the mice were anesthetized



with ether, their abdominal cavities were opened, the pylorus was ligated, and the incisions were quickly sutured. After a duration of 3 hours, the mice were euthanized, and their abdominal cavities were reopened. A sample of 1 milliliter of gastric juice was collected and placed into a 50 mL conical flask, after which 0.05 mol/L hydrochloric acid solution was added and thoroughly mixed. The resulting solution was then divided into two new protein tubes, which were tightly sealed and incubated at 37°C for 24 h. Once the incubation was complete, the protein tubes were removed, and the lengths (mm) of the clear sections at each end of the tubes were measured using a Vernier caliper. Pepsin activity and pepsin secretion were calculated.

Pepsin activity (U/mL) = (the average length of the clear section of the four terminal tubes) $\times 2 \times 16$

Pepsin discharge (U/h) = pepsin activity $\times$ volume of gastric juice produced per hour.

ETHANOL-INDUCED GASTRIC MUCOSAL DAMAGE

All the mice except those in the control group were given 1 ml absolute ethanol 1 hr after the last administration of drugs. Subsequently, all animals were anesthetized. Next, the stomachs were removed and opened along the long curvature to observe the lesions macroscopically 1 hr after being injected with 6 ml of 4% paraformaldehyde. Scoring standard was as follows: Intact gastric mucosa was scored as 0; point-shaped bleeding, point-shaped erosion, and strip-shaped lesion of no more than 1 mm were all scored as 1; strip-shaped lesion with more than 1 mm was doubly scored. Total scores of every mice were considered as the lesion index

Inhibition ratio of lesion formation (%) = $(A - B)/A \times 100\%$

(A, B were the lesion indices of the model group and drug-treated group, respectively)

WESTERN BLOT ANALYSIS

To determine the stomach protein level of neuronal nitric oxide synthase (nNOS), stomach tissue were prepared in the RIPA lysis buffer. The protein were

denatured by boiling for 5 min, and was then separated by 10% polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes. After blocking in 3% BSA for 1 h, the membranes were probed with primary antibodies such as nNOS (1:1000, ab76067) or β -actin (1:1000, MA5-11869) overnight at 4 °C. The membranes were washed and incubated with HRP-conjugated antirabbit (1:2500, against nNOS) or anti-mouse antibody (1:2500, against β -actin) for 40 min. The protein were visualized using an enhanced chemiluminescence (ECL) advanced kit. The protein expression was observed using as FUSION Solo System. The protein expression were semi quantified using Image

ENZYME LINKED IMMUNOSORBENT ASSAY

Blood samples from mice were gathered, and the serum was isolated following centrifugation at 3000 g for 15 minutes at 4°C. The samples were preserved at -80°C for subsequent analysis. Serum levels of 5-HT were determined with a Serotonin Enzyme Linked Immunosorbent Assay (ELISA) Kit (Avantor, Jaipur).

HISTOPATHOLOGY

Balb/C mice were sacrificed by cervical decapitation on 14th day, the gastric tissues were removed, washed with saline and fixed with 4% formalin. The gastric tissue sections were obtained, stained with hematoxylin-eosin stain (HE stain) and viewed under 100X magnification.

STATISTICAL ANALYSIS

All data are expressed as the mean $\pm$ standard deviation (SD). The statistical significance were analyzed by one-way analysis of variance (ANOVA), followed by post hoc multiple comparisons with Fisher's LSD t-test using GraphPad Prism version 10.6. The differences observed were considered statistically significant at $p < 0.05$ or 0.01

RESULTS

ACUTE ORAL TOXICITY (AOT) TEST



The findings of the acute oral toxicity assessment of Kaempferol-4-O- β -D-glucopyranoside (K-4-O- β -D-g) showed a distinct dose-dependent toxic response. In the initial hours post-administration, the treated animals displayed clinical symptoms such as reduced locomotor activity, ataxia, and, at elevated doses, signs of lethargy and hypothermia. Mortality began to occur at the 1,000 mg/kg dosage, reaching a 100% fatality rate at 2,000 mg/kg (Table 7.2). The estimated median lethal dose (LD₅₀) was found to be 1,311.45 mg/kg, with a 95% confidence interval from 1,037.8 to 1,657.27 mg/kg.

No statistically significant variations in average body weights were observed between the control and treated groups at lower doses, although the higher doses affected weight gain. In summary, the results suggest that while doses beneath the LD₅₀ are relatively tolerated, the extract demonstrates moderate acute oral toxicity at elevated levels, accompanied by notable clinical and behavioral effects.

BODY WEIGHT, FOOD, WATER CONSUMPTION, AND BEHAVIORAL CHANGES IN MICE

During the adjustment phase, there were no significant differences in weight, food intake, or water consumption among the mice. After administering loperamide, the body weight of the mice decreased significantly compared to the control group. However, pretreatment with a low dose of 50 mg/kg of K-4-O- β -D-g showed a moderate increase in weight, while a high dose of 100 mg/kg of K-4-O- β -D-g resulted in a significant increase in body weight ($p < 0.01$ as shown in Figure 1). Additionally, treatment with mosapride led to a notable increase in weight.

Behavioral changes were significant in the loperamide group; however, treatment with 50 mg/kg K-4-O- β -D-g showed improvement in these changes.

In contrast, treatment with 100 mg/kg K-4-O- β -D-g and mosapride returned the results to baseline.

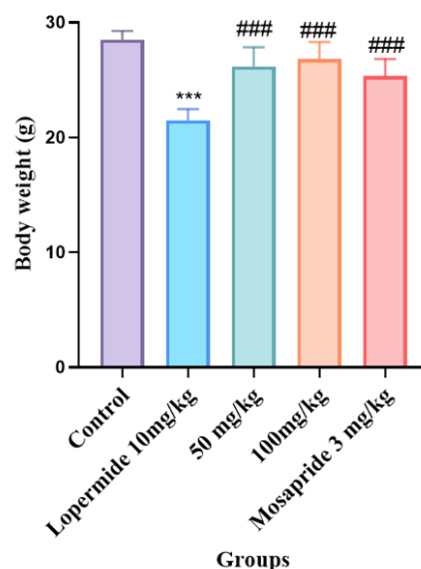


Figure 1 Results of K-4-O- β -D-g on body weight.

The body weight changes checked (A) The data are organized as the mean $\pm$ SD. * $p < 0.05$, *** $p < 0.001$, for the Control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ for the loperamide group.

EFFECT ON DELAYED GASTRIC EMPTYING

Loperamide injection caused food to be retained in the stomach, while prior treatment with K-4-O- β -D-g reduced this effect, as evidenced by macroscopic observations (Figure 2). This result was confirmed through quantitative analysis. Both K-4-O- β -D-g treated groups showed significantly lower gastric weight compared to the loperamide group, with the 50 mg/kg of K-4-O- β -D-g dose producing a more pronounced reduction ($p < 0.05$). Similarly, pretreatment with K-4-O- β -D-g led to a dose-dependent decrease in the amount of phenol red retained in the stomach, with the 100 mg/kg of K-4-O- β -D-g showing superior improvement. Pretreatment with mosapride produced effects comparable to those observed with the higher K-4-O- β -D-g dose ($p < 0.01$ as shown in Figure 2).



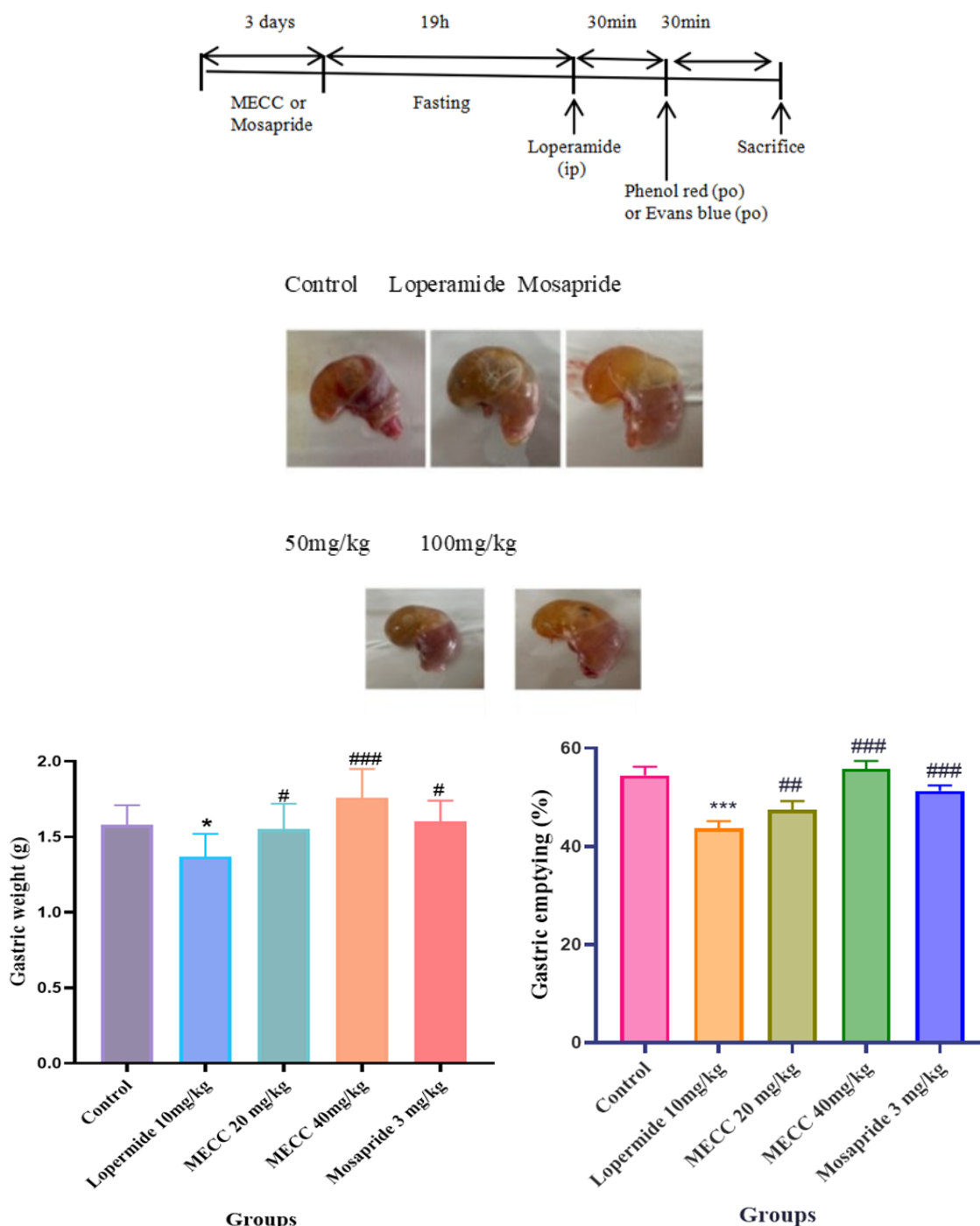


Figure 2 Results of K-4-O-β-D-g on gastric emptying. The experimental schedule is summarized in (A). For 3 days, mice (n = 6/group) were treated by po with 50, and 100 mg/kg of K-4-O-β-D-g or 3 mg/kg of mosapride and then treated by IP injection with 10 mg/kg of loperamide. After the treatment of phenol red, results of visualization (B), weight of stomach (C), and results of gastric emptying (D) are presented. The data are organized as the mean ± SD. * $p < 0.05$, *** $p < 0.001$, for the Control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ for the loperamide group.

EFFECT ON INTESTINAL MOTILITY

Looperamide administration markedly reduced motility in the small intestine compared to the control group. This reduction was moderately reversed by

pretreatment with 50 mg/kg of K-4-O-β-D-g, while a more pronounced restoration of motility was observed with 100 mg/kg of K-4-O-β-D-g ($p < 0.01$). Furthermore, pretreatment with mosapride notably restored small intestine motility, showing effects



comparable to those seen with 100 mg/kg of K-4-O- β -D-g ($p < 0.01$ as shown in Figure 3).



(A) Control (B) Loperamide (C) K-4-O- β -D-g 50mg/kg (D) K-4-O- β -D-g 100mg/kg (E) Mosapride

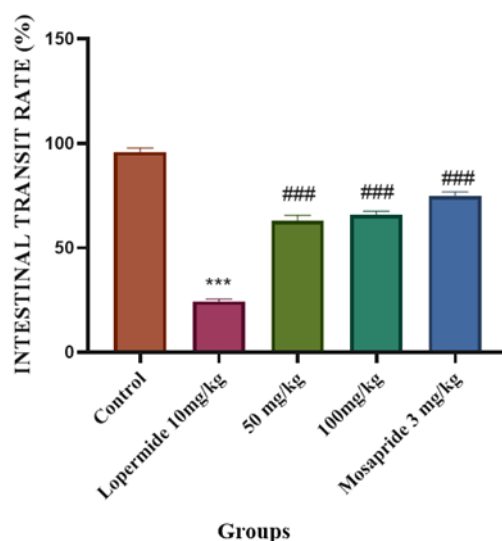


Figure 3 Results of K-4-O- β -D-g on small intestinal motility. For 3 days, mice ($n = 6/\text{group}$) were treated by po with 50 and 100 mg/kg of K-4-O- β -D-g or 3 mg/kg of mosapride and then treated by IP injection with 10 mg/kg of loperamide. After 30 min of treatment with Evans blue, the distances stained were checked (A) and quantified (B). The data are organized as the mean $\pm$ SD. *** $p < 0.001$ for the Control group; ### $p < 0.001$ for the loperamide group.

EFFECT ON ISOLATED GASTRIC SMOOTH MUSCLE

The spontaneous contractile activity in strips of gastric smooth muscle was reduced in FD mice, demonstrating a decline in frequency while the amplitude remained unchanged. Importantly, administration of K-4-O- β -D-g groups (50 and 100 mg/kg) and mosapride enhanced the frequency of spontaneous gastric contractions in FD mice ($p < 0.01$). The control group displayed normal motility. A dose-dependent increase in the motility index (MI)

was observed, indicating that higher doses led to stronger contractions and lower EC₅₀ values, signifying greater potency in the K-4-O- β -D-g groups (50 and 100 mg/kg). A reduced MI corresponds with its known inhibitory effect on gastric motility, while a higher EC₅₀ reflects a weaker stimulation of contractions in the loperamide group. An increased MI signifies a prokinetic (motility-enhancing) effect, and a moderate EC₅₀ suggests effective stimulation of contractions in the mosapride group similar to the K-4-O- β -D-g groups (50 and 100 mg/kg) ($p < 0.01$ as shown in Figure 3).

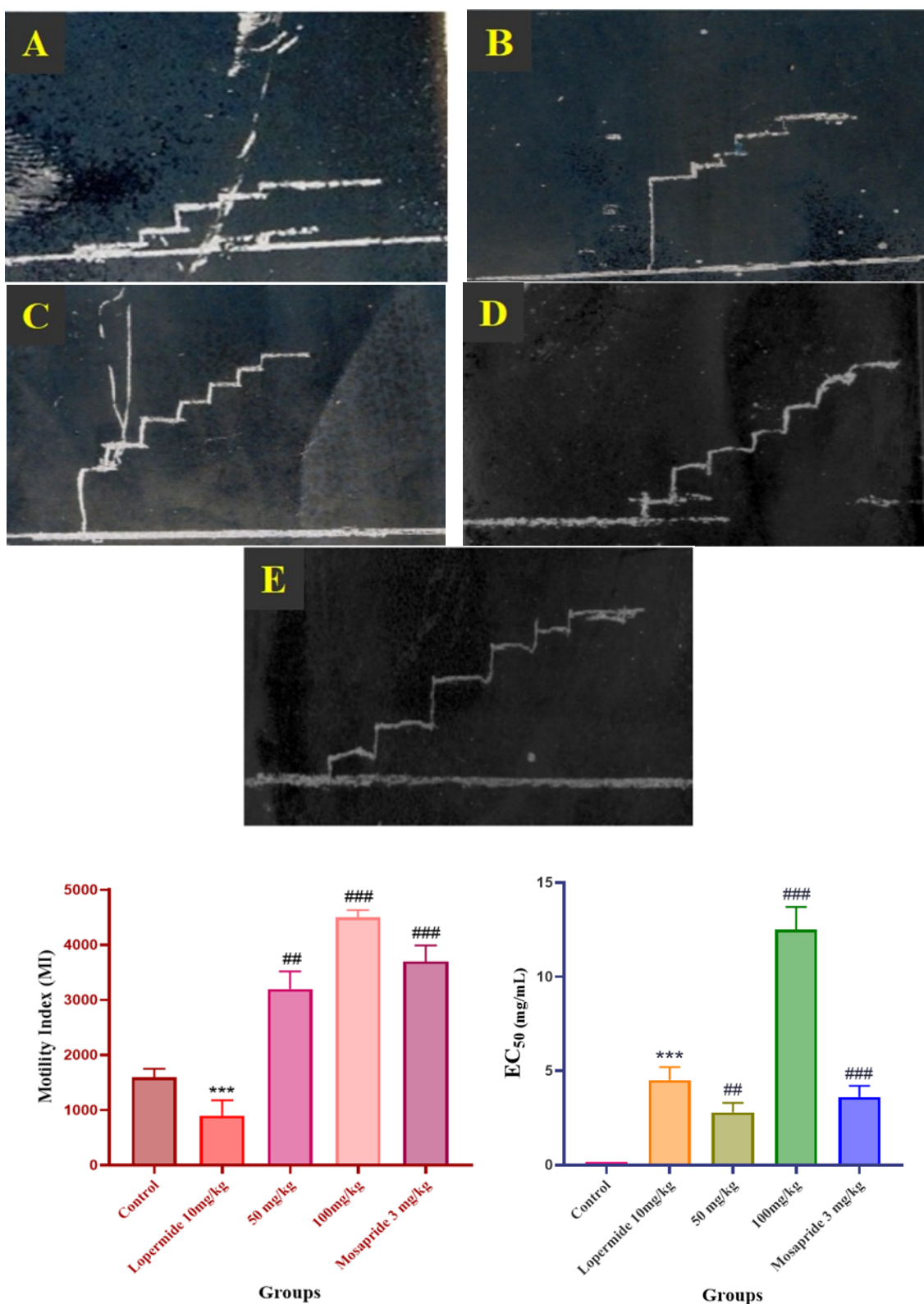


Figure 3 Results of K-4-O-β-D-g on gastric smooth muscle. The spontaneous contractile curve of gastric smooth muscle strips are shown as Control (A), Loperamide 10mg/kg (B), 50 mg/kg K-4-O-β-D-g (C), 100mg/kg K-4-O-β-D-g (D), mosapride 3mg/kg (E) and the results of motility index (F) and concentration at 50% of maximal effect (EC₅₀) of Ach (G) were represented. The data are organized as the mean ± SD. *** p < 0.001 for the Control group; ### p < 0.001 for the loperamide group.

EFFECT ON DIGESTIVE ENZYMES

Compared with the control group, the volumes of gastric juice, as well as pepsin activity and pepsin excretion over 1 hour, were notably reduced in the loperamide group. Pretreatment with mosapride and

50 mg/kg of K-4-O- β -D-g moderately increased pepsin excretion compared to the loperamide group, while 100 mg/kg of K-4-O- β -D-g resulted in a considerably greater enhancement of pepsin excretion ($p < 0.01$ as shown in Figure 4).

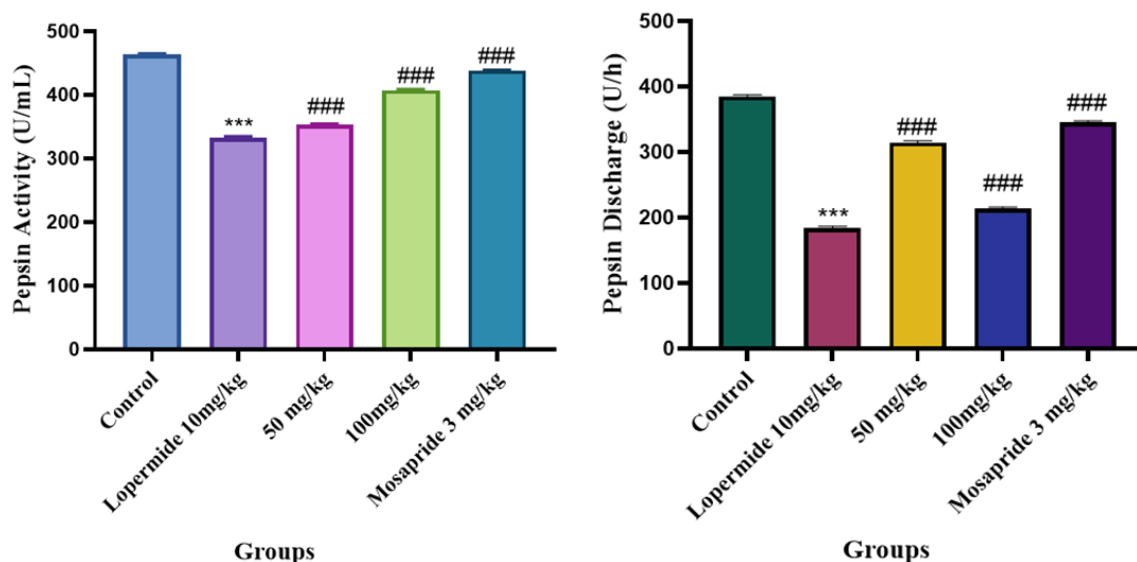


Figure 4 Results of K-4-O- β -D-g on digestive enzymes. The analyses of pepsin activity (A) and pepsin excretion (B) were measured and quantified. The data are organized as the mean $\pm$ SD. * $p < 0.001$ for the Control group; ### $p < 0.001$ for the loperamide group.**

EFFECT ON GASTRIC MUCOSAL DAMAGE

Compared with the control group, mice treated with loperamide exhibited significant damage to their gastric mucosa, including shedding, bleeding, and an influx of inflammatory cells ($p < 0.01$). In contrast, the gastric mucosa of mice pretreated with 50 mg/kg of K-4-O- β -D-g showed milder damage and reduced

inflammatory cell infiltration, while pretreatment with 100 mg/kg of K-4-O- β -D-g and mosapride resulted in a notably lighter mucosal appearance and further diminished inflammation ($p < 0.01$). These findings suggest that K-4-O- β -D-g reduces gastric mucosal inflammation and promotes its healing in a dose-dependent manner ($p < 0.01$ as shown in Figure 5)

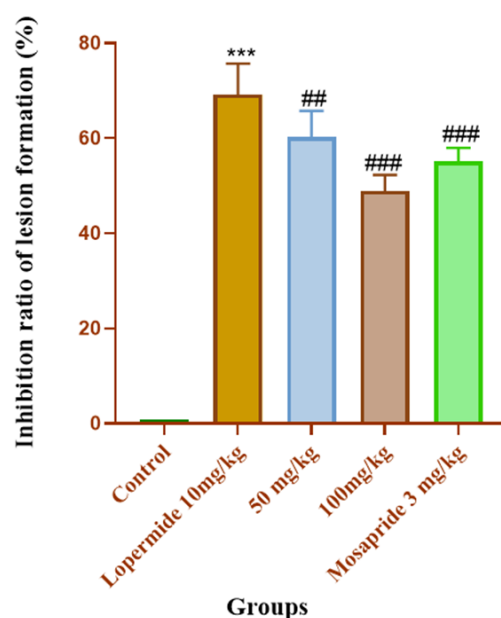
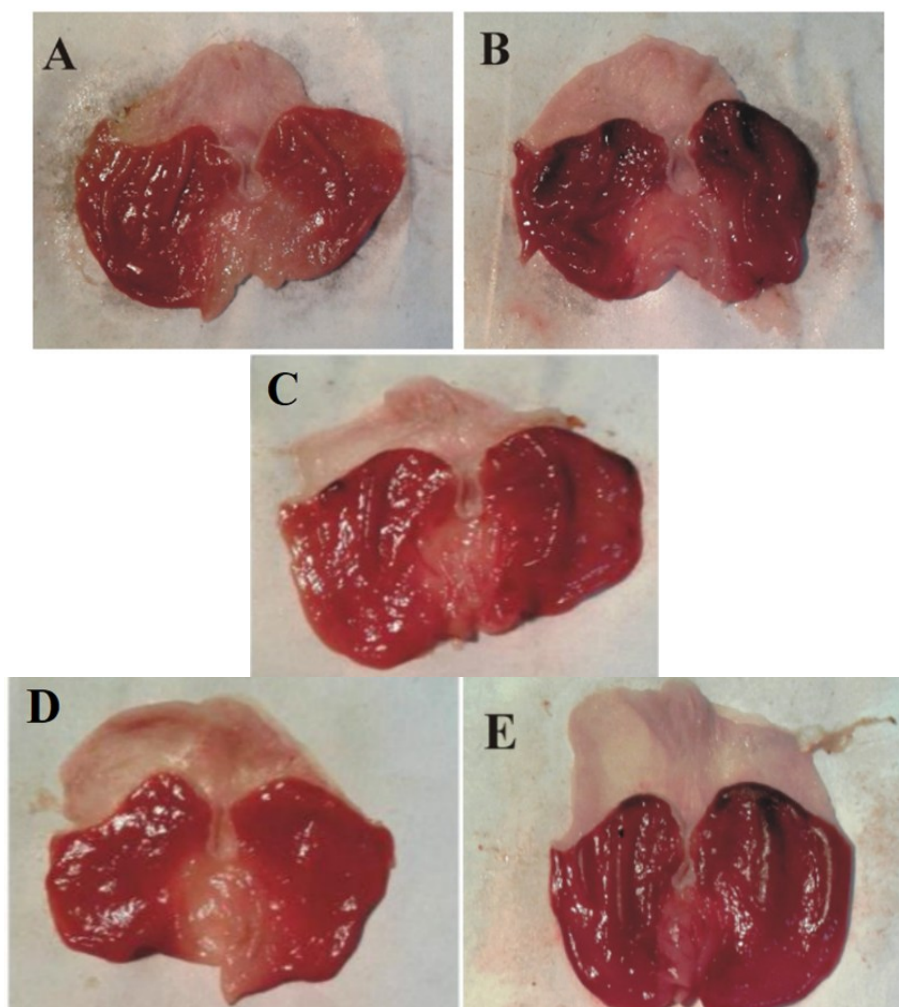


Figure 5 Results of protection K-4-O-β-D-g on gastric mucosal injury. The experimental schedule is administration of 1 ml absolute ethanol 1 hr after the last administration of drugs. After that, results of visualization shows Control (A), Loperamide 10mg/kg (B), 50 mg/kg K-4-O-β-D-g (C), 100mg/kg K-4-O-β-D-g (D), mosapride 3mg/kg (E) and results of inhibition ratio of lesion formation represented (F).

The data are organized as the mean $\pm$ SD. *** $p < 0.001$ for the Control group; ## $p < 0.01$, ### $p < 0.001$ for the loperamide group.

EFFECT ON MOLECULES ASSOCIATED WITH GASTROINTESTINAL MOTILITY

Loperamide injection significantly reduced the expression of nNOS, TMEM16A, and TRPM7 proteins in gastric tissue, while prior treatment with K-4-O- β -D-g led to a substantial increase in nNOS, TMEM16A, and TRPM7 protein expression ($p < 0.05$ as shown in Figure 6). Additionally, loperamide

injection lowered the expression of smooth muscle contraction-related genes, such as 5HT4R, RYR3, ANO1, and smMLCK ($p < 0.01$). These effects were reversed by pretreatment with 50 mg/kg of K-4-O- β -D-g and 100mg/kg K-4-O- β -D-g. Mosapride positively influenced the expression of nNOS, TMEM16A, and TRPM7 proteins, as well as the gene expression of 5HT4R, RYR3, ANO1, and smMLCK.

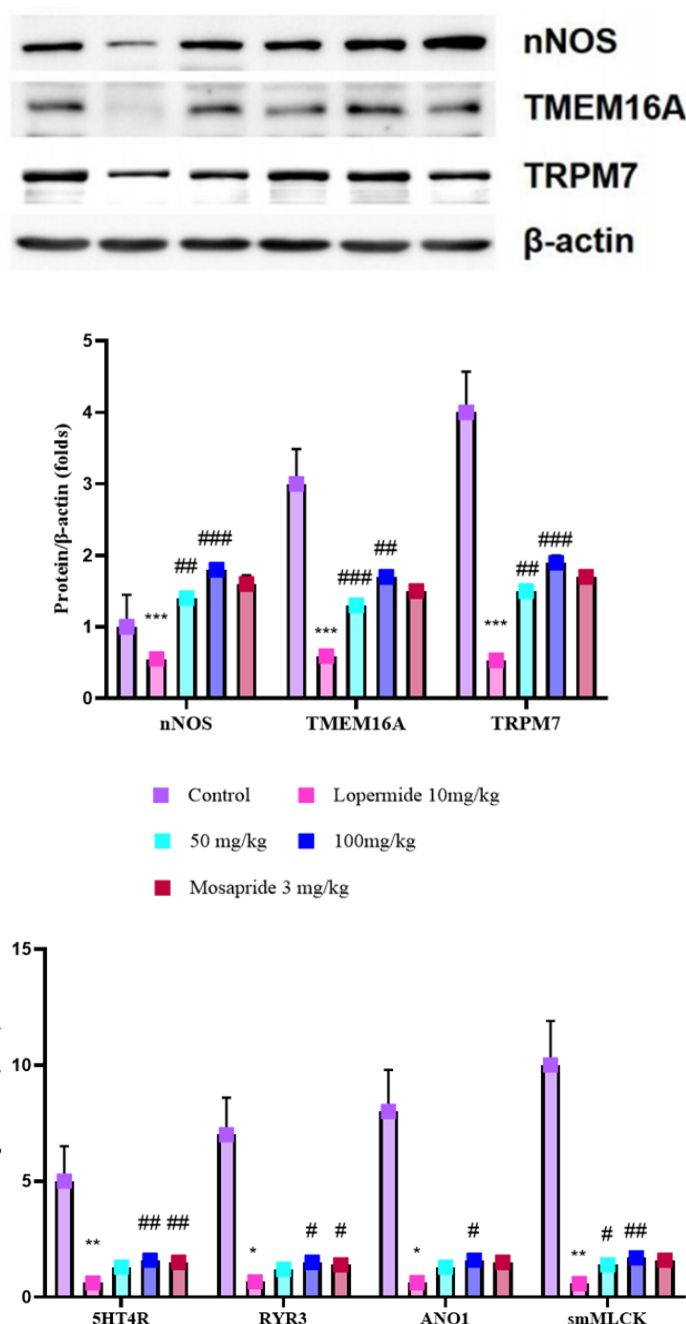


Figure 6 Results of K-4-O- β -D-g on GI motility-associated molecules in stomach tissue. The analyses of Western blot for nNOS, TMEM16A, and TRPM7 (A) and semi-quantifications (B) were conducted ($n = 3$). The analyses of mRNA expression of GI motility-associated genes were performed (C) ($n = 3$) in the stomach tissue. The data are organized as the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for the Control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ for the loperamide group.



K-4-O- β -D-G PROMOTED THE RELEASE OF SERUM 5-HT

Reduced levels of serum 5-HT were seen in the loperamide group, but these levels were enhanced in animals that received pretreatment with 50 mg/kg of

K-4-O- β -D-g, 100 mg/kg of K-4-O- β -D-g and mosapride ($p < 0.01$). This further indicates that the 5-HT system plays a role in the mechanism by which K-4-O- β -D-g aids in alleviating gastric dysmotility and hypersensitivity in FD mice (Figure 7).

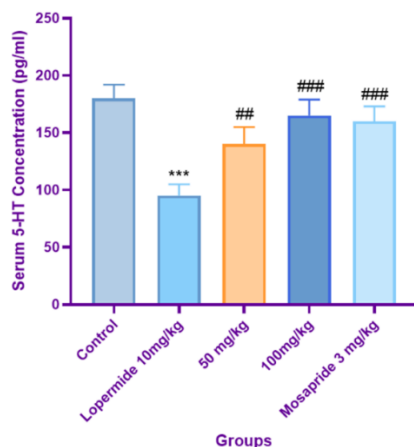


Figure 7 Results of K-4-O- β -D-g on serum 5-HT levels. The concentration of 5-HT in serum (A) was analyzed. Data are presented as the mean $\pm$ SD. * $p < 0.001$ compared to Control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared to loperamide group.**

HISTOPATHOLOGICAL EVALUATION

In the loperamide group, notable alterations were documented, including a striking thickening of the mucosal layer, a prominent infiltration of inflammatory cells, and sloughing of the mucosa that revealed underlying damage. The tissues showed signs of necrosis and mild hemorrhage, painting a picture of distress. In stark contrast, the control group

exhibited a well-preserved tissue architecture, showcasing vibrant and healthy mucosal surfaces. Remarkably, pretreatment with K-4-O- β -D-g and mosapride led to a considerable reduction in the extent of damage, with visibly less tissue necrosis and hemorrhage (Figure 8). This intervention effectively shielded the tissues, highlighting its protective capabilities against the adverse effects observed in the loperamide group.

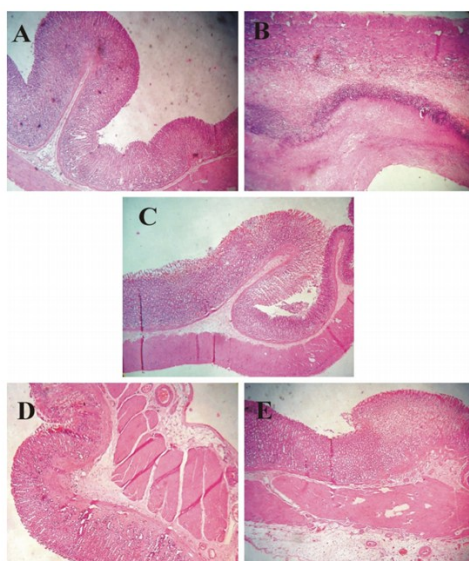


Figure 8 Effect of K-4-O- β -D-g on histology of stomach

Histological sections of stomach from vehicle treated control group (A) showing normal architecture. Stomach section from positive control (B) that received loperamide shows severe thickened mucosa, increased infiltration of inflammatory cells, tissue necrosis and hemorrhage as compared to control group. Section of stomach from loperamide (10mg/kg) treated mice which concurrently received K-4-O- β -D-g (50 mg/kg) (C), K-4-O- β -D-g (100 mg/kg) (D), mosapride (3mg/kg) (E) respectively orally for seven days shows a marked decrease in infiltration of inflammatory cells, edema, sloughing of mucosa, tissue necrosis and hemorrhage and indicates the prevention of the damage compared to loperamide treated group

DISCUSSION

Citrullus colocynthis, a sprawling herb of the Cucurbitaceae family, has long been used in traditional medicine to treat diverse ailments including fever, parasitic infections, liver and abdominal disorders, edema, tumors, and gastrointestinal problems. It exhibits purgative, anti-diabetic, anti-neoplastic, anti-rheumatic, and anti-allergic effects, supported by identification of 75 phytochemicals. Modern pharmacology confirms its insecticidal, cytotoxic, anti-diabetic, immunostimulatory, antimicrobial, anti-inflammatory, antioxidative, and gastrointestinal motility-enhancing activities, linking traditional uses with contemporary evidence (Cheng et al. 2023; Afzal et al. 2023). Phytochemical analyses reveal flavonoids such as quercetin, kaempferol, and kaempferol-4-O- β -D-glucopyranoside (K-4-O- β -D-g) as key bioactive compounds mediating anti-inflammatory, antioxidative, and motility-improving effects, validating traditional gastrointestinal applications (Afzal et al. 2023). Using a loperamide-induced mouse model mimicking functional dyspepsia (FD), K-4-O- β -D-g at 50 and 100 mg/kg doses significantly improved symptoms by enhancing gastric emptying and intestinal transit as evidenced by reduced phenol red dye retention and increased Evans blue dye progression, comparable to the prokinetic mosapride (Choi et al. 2023).

K-4-O- β -D-g also restored pepsin secretion impaired by loperamide, indicating improved gastric secretory

function (Shao et al. 2022). Molecular studies showed normalization of neuronal nitric oxide synthase (nNOS) levels and upregulation of motility-related genes including 5-HT₄ receptor, ryanodine receptor 3, anoctamin-1, and smooth muscle myosin light chain kinase, thereby restoring inhibitory neurotransmission, smooth muscle contractility, and interstitial cells of Cajal pacemaking necessary for coordinated motility (Choi et al. 2023; Xiao et al. 2022).

Ex vivo studies on gastric muscle strips revealed increased contraction frequency and amplitude and improved responsiveness to cholinergic and serotonergic stimuli, alongside elevated serum serotonin levels, elucidating multifaceted motility-enhancing mechanisms of K-4-O- β -D-g (Xiao et al. 2022). Histopathology demonstrated protection against loperamide and ethanol-induced gastric mucosal damage, reducing inflammation and necrosis, indicating cytoprotective and anti-inflammatory effects. Overall, K-4-O- β -D-g from *Citrullus colocynthis* acts through multiple pathways to alleviate FD pathology, improving motility, secretion, and mucosal integrity while modulating neuronal and muscular factors. Despite promising results, the reliance on an acute pharmacological model highlights the need for further studies on chronic effects, dose optimization, safety, and broader molecular interactions. This compound shows strong potential as a multitarget therapeutic for functional dyspepsia.

CONCLUSION

The methanolic extract of *Citrullus colocynthis* fruit, rich in phenols and flavonoids, yielded three compounds: Quercetin, Kaempferol, and the novel Kaempferol-4-O- β -D-glucopyranoside (K-4-O- β -D-g). Pharmacological studies in loperamide-induced functional dyspepsia mice showed that K-4-O- β -D-g improved gastric emptying, intestinal motility, gastric contractility, and pepsin secretion. Histopathology confirmed reduced stomach inflammation. The compound increased levels of nNOS, TMEM16A, TRPM7, and genes involved in smooth muscle contraction (5HT₄R, RYR3, ANO1, smMLCK), as well as serum serotonin, contributing to relief from gastric dysmotility and hypersensitivity. Thus, K-4-



O-β-D-g from *Citrullus colocynthis* holds promise as a complementary treatment for functional dyspepsia.

ACKNOWLEDGEMENTS

The study was supported by Pallavan Pharmacy college, Kanchipuram.

REFERENCE

1. Afzal M, Khan AS, Zeshan B, Riaz M, Ejaz U, Saleem A, et al. Characterization of bioactive compounds and novel proteins derived from promising source *Citrullus colocynthis* along with in-vitro and in-vivo activities. *Molecules*. 2023;28(4):1743.
2. Agah S, Akbari A, Heshmati J, Sepidarkish M, Morvaridzadeh M, Adibi P, et al. Systematic review with meta-analysis: Effects of probiotic supplementation on symptoms in functional dyspepsia. *J Funct Foods*. 2020;68:103902.
3. Amerikanou C, Kleftaki SA, Valsamidou E, Chroni E, Biagki T, Sigala D, et al. Food, dietary patterns, or is eating behavior to blame? Analyzing the nutritional aspects of functional dyspepsia. *Nutrients*. 2023;15(6):1544.
4. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. *Life Sci*. 2005;78:431–41.
5. Benzie IF, Wachtel-Galor S, editors. *Herbal medicine: biomolecular and clinical aspects*. Boca Raton: CRC Press; 2011.
6. Black CJ, Paine PA, Agrawal A, Aziz I, Eugenicos MP, Houghton LA, et al. British Society of Gastroenterology guidelines on the management of functional dyspepsia. *Gut*. 2022;71(9):1697–723.
7. Boeing T, de Souza P, da Silva LM, Junior AG. Herbal medicines in the treatment of dyspepsia: an overview. *Planta Med*. 2022;88(8):664–77.
8. Bousdouni P, Kandyliari A, Koutelidakis AE. Probiotics and phytochemicals: role on gut microbiota and efficacy on IBS, functional dyspepsia, and constipation. *Gastrointest Disord*. 2022;4(1):5.
9. Chawech R, Mhalla D, Trigui M, Mihoubi M, Fabre N, Jarraya R. Chemical composition and antibacterial activity of extracts and compounds isolated from *Citrullus colocynthis*. *J Pharmacogn Phytochem*. 2015;4(4):197.
10. Cheng X, Qin M, Chen R, Jia Y, Zhu Q, Chen G, et al. *Citrullus colocynthis* (L.) Schrad.: a promising pharmaceutical resource for multiple diseases. *Molecules*. 2023;28(17):6221.
11. Choi NR, Jung D, Kim SC, Park JW, Choi WG, Kim BJ. Analysis of network pharmacological efficacy and therapeutic effectiveness in animal models for functional dyspepsia of *Foeniculi fructus*. *Nutrients*. 2023;15(12):2644.
12. Dhakad PK, Sharma PK, Kumar S. A review on phytochemical studies and biological potential of *Citrullus colocynthis*. *J Bioeng Biosci*. 2017;5(4):55–64.
13. Fărcaș RA, Grad S, Grad C, Dumitrașcu DL. Microbiota and digestive metabolites alterations in functional dyspepsia. *J Gastrointest Liver Dis*. 2024;33(1).
14. Francis P, Zavala SR. Functional dyspepsia. In: *StatPearls* [Internet]. Treasure Island: StatPearls Publishing; 2024.
15. Hameed B, Ali Q, Hafeez MM, Malik A. Antibacterial and antifungal activity of fruit, seed and root extracts of *Citrullus colocynthis*. *Biol Clin Sci Res J*. 2020;33:1–5.
16. Harmon RC, Peura DA. Evaluation and management of dyspepsia. *Ther Adv Gastroenterol*. 2010;3(2):87–98.
17. Hasan H, Akrim Z, Shuib F, Abdraba D. Phytochemical investigation and exploring *Citrullus colocynthis* extracts as antibacterial agents against some Gram-positive and Gram-negative species. *AlQalam J Med Appl Sci*. 2025;392–400.
18. Heading RC. Definitions of dyspepsia. *Scand J Gastroenterol*. 1991;26(sup182):1–6.
19. Hussain AI, Rathore HA, Sattar MZ, Chatha SA, Ahmad F, Ahmad A, et al. Phenolic profile and antioxidant activity of various extracts from *Citrullus colocynthis*. *Ind Crops Prod*. 2013;45:416–22.
20. Hwang SJ, Wang JH, Lee JS, Lee HD, Choi TJ, Choi SH, et al. Yeokwisan enhances gastric emptying via modulation of the ghrelin pathway in functional dyspepsia mice. *Front Pharmacol*. 2021;12:753153.
21. Idan SA, Al-Marzoqi AH, Hameed IH. Spectral analysis and antibacterial activity of methanolic fruit extract of *Citrullus colocynthis*. *Afr J Biotechnol*. 2015;14(46):3131–58.



22. Jeon YJ, Lee JS, Cho YR, Lee SB, Kim WY, Roh SS, et al. Banha-sasim-tang improves gastrointestinal function in loperamide-induced functional dyspepsia mouse model. *J Ethnopharmacol.* 2019;238:111834.
23. Kapoor M, Kaur N, Sharma C, Kaur G, Kaur R, Batra K, et al. *Citrullus colocynthis*: an important plant in Indian traditional medicine. *Pharmacogn Rev.* 2020;14(27).
24. Khakisahneh S, Wang JH, Zhang XY, Choi Y, Han SY, Song EJ, et al. Comparative evaluation of two herbal formulas for gastrointestinal function and gut microbiota modulation in loperamide-induced dyspepsia rats. *Sci Rep.* 2025;15(1):31186.
25. Kim SH, Choi Y, Oh J, Lim EY, Lee JE, Song EJ, et al. Associations among the duodenal ecosystem, gut microbiota, and nutrient intake in functional dyspepsia. *Gut Liver.* 2023;18(4):621.
26. Koduru P, Irani M, Quigley EM. Definition, pathogenesis, and management of dyspepsia. *Clin Gastroenterol Hepatol.* 2018;16(4):467–79.
27. Kumar D, Singh L, Antil R, Kumari S, Dahiya P. GC-MS analysis and phytochemical screening of methanolic fruit extract of *Citrullus colocynthis*. *J Pharmacogn Phytochem.* 2019;8(3):3360–3.
28. Kumar S, Kumar D, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* methanolic fruit extract. *Acta Pharm.* 2008;58(2):215–20.
29. Li QY, Munawar M, Saeed M, Shen JQ, Khan MS, Noreen S, et al. *Citrullus colocynthis*: promising traditional uses, pharmacological effects and potential applications. *Front Pharmacol.* 2022;12:791049.
30. Luo L, Hu M, Li Y, Chen Y, Zhang S, Chen J, et al. Association between metabolic profile and microbiomic changes in rats with functional dyspepsia. *RSC Adv.* 2018;8(36):20166–81.
31. Madisch A, Holtmann G, Mayr G, Vinson B, Hotz J. Treatment of functional dyspepsia with a herbal preparation: a double-blind, randomized, placebo-controlled trial. *Digestion.* 2004;69(1):45–52.
32. Mahadeva S, Goh KL. Epidemiology of functional dyspepsia: a global perspective. *World J Gastroenterol.* 2006;12(17):2661.
33. Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A, Aouni M. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis*. *J Ethnopharmacol.* 2010;128(1):15–9.
34. Mounsey A, Barzin A, Rietz A. Functional dyspepsia: evaluation and management. *Am Fam Physician.* 2020;101(2):84–8.
35. Mukhtar HM, Singh H. Pharmacological and phytochemical updates on *Citrullus colocynthis* and *Citrullus lanata*. *Indo Glob J Pharm Sci.* 2020;10(4):10–8.
36. Na’Allah A, Bashir S, Alabi M, Kareem FA, Olaifa GO, Afolabi FJ, et al. Anti-diarrhoea properties of ethanol extract of *Citrullus colocynthis* fruit pulp: In vivo and in silico studies. *Adv Tradit Med.* 2025;25(1):269–85.
37. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. *Nat Prod Rep.* 2000;17:215–34.
38. Pradhan S, Goswami S, Manna S, Adak T, Pradhan A, Dolui S, et al. Phytochemical analysis of *Citrullus colocynthis* and its antibacterial activity. *J Pharmacogn Phytochem.* 2023;12(3):156–9.
39. Ramakrishna D, Suvarchala V, Chaitanya G, Shasthree T. Preliminary phytochemical screening of *Citrullus colocynthis*. *Res J Chem Environ.* 2019;23(11):41–55.
40. Rösch W, Liebrechts T, Gundermann KJ, Vinson B, Holtmann G. Phytotherapy for functional dyspepsia: a review of clinical evidence for STW 5. *Phytomedicine.* 2006;13:114–21.
41. Samimi S, Nimrouzi M, Zarshenas MM, Fallahzadeh E, Vardanjani HM, Sadeghi E, et al. The efficacy of a traditional herbal compound for functional dyspepsia: a randomized, double-blind, placebo-controlled trial. *Jundishapur J Nat Pharm Prod.* 2024;19:e148163.
42. Shaikh J, Shaikh D, Rahman AB, Shafi S. Antimicrobial and toxicological studies on fruit pulp of *Citrullus colocynthis*. *Pak J Pharm Sci.* 2016;29(1):9–16.
43. Shao Z, Zhong J, Fang Y, Ma Y. Effect of Kvass on improving functional dyspepsia in rats. *Comput Math Methods Med.* 2022;2022:5169892.
44. Sharma DK, Jain VK. Therapeutic, ethnomedicinal and pharmacological perspective



- of *Citrullus colocynthis*. *Cent Asian J Med Nat Sci*. 2022;3(4):260–7.
45. Soufane S, Bedda A, Mahdeb N, Bouzidi A. Acute toxicity study on *Citrullus colocynthis* fruit methanol extract in albino rats. *J Appl Pharm Sci*. 2013;3(6):88–93.
46. Swami OC, Shah NJ. Functional dyspepsia and the role of digestive enzyme supplements in therapy. *Int J Basic Clin Pharmacol*. 2017;6(5):1035.
47. Swarnakar G, Kumawat A, Menaria K, Chouhan HS. Pharmacological studies of medicinal plant *Citrullus colocynthis*. A.
48. Tack J, Bisschops RAF, Sarnelli G. Pathophysiology and treatment of functional dyspepsia. *Gastroenterology*. 2004;127(4):1239–55.
49. Thamer FH, Thamer N, Alhamzi A, Al-Ansi N, Al-Sadi S, Al-Shibeh A. Antioxidant capacity, total phenol contents and phytochemical screening of *Citrullus colocynthis* extracts. *Am J Biochem Biotechnol*. 2023;19(1):12–9.
50. Tziatzios G, Gkolfakis P, Papanikolaou IS, Mathur R, Pimentel M, Giamarellos-Bourboulis EJ, et al. Gut microbiota dysbiosis in functional dyspepsia. *Microorganisms*. 2020;8(5):691.
51. Uma C, Sekar KG. Phytochemical analysis of *Citrullus colocynthis* (bitter apple). *J Pharmacogn Phytochem*. 2014;2(6).
52. Wagner H. Multitarget therapy: the future of treatment for functional dyspepsia. *Phytomedicine*. 2006;13:122–9.
53. Wei L, Singh R, Ro S, Ghoshal UC. Gut microbiota dysbiosis in functional gastrointestinal disorders. *JGH Open*. 2021;5(9):976–87.
54. Xiao Z, Xu J, Tan J, Zhang S, Wang N, Wang R, et al. Zhizhu Kuanzhong alleviates gastric hypersensitivity and motor dysfunction in functional dyspepsia rats. *Front Pharmacol*. 2022;13:1026660.
55. Yamawaki H, Futagami S, Wakabayashi M, Sakasegawa N, Agawa S, Higuchi K, et al. Management of functional dyspepsia: state of the art and emerging therapies. *Ther Adv Chronic Dis*. 2018;9(1):23–32.
56. Ye Y, Wang XR, Zheng Y, Yang JW, Yang NN, Shi GX, et al. Choosing an animal model for the study of functional dyspepsia. *Can J Gastroenterol Hepatol*. 2018;2018:1531958.
57. Zhang S, Lin L, Liu W, Zou B, Cai Y, Liu D, et al. Shen-Ling-Bai-Zhu-San alleviates functional dyspepsia in rats and modulates gut microbiota. *Nutr Res*. 2019;71:89–99.
58. Zhong LY, Tong HL, Zhu J, Lv M. Pharmacological effects of different ginger juices on concurrent symptoms in animal models of functional dyspepsia. *Food Sci Nutr*. 2019;7(7):2205–13.
59. Zhou J, Li S, Wang Y, Lei Y, Foreman RD, Yin J, et al. Effects and mechanisms of auricular electroacupuncture on gastric hypersensitivity in a rodent model of functional dyspepsia. *PLoS One*. 2017;12(3):e0174568.
60. Zhou L, Zeng Y, Zhang H, Ma Y. The role of gastrointestinal microbiota in functional dyspepsia: a review. *Front Physiol*. 2022;13:910568.
61. Zhu J, Tong H, Ye X, Zhang J, Huang Y, Yang M, et al. Effects of low-dose and high-dose decoctions of *Fructus aurantii* in a rat model of functional dyspepsia. *Med Sci Monit*. 2020;26:e919815.

HOW TO CITE: Hemalatha, Swarnalatha, Karthi, Experimental Investigation on the Therapeutic Potential of Kaempferol-4-O-B-D-glucopyranoside from *Citrullus colocynthis* in the Management of Functional Dyspepsia, *J. Pharm. Sci.*, 2025, 1 (11), 866-882. <https://doi.org/10.5281/zenodo.17622691>

