Effect of Two Mucoprotectants, Gelatin Tannate and Xyloglucan plus Gelatin, on Cholera Toxin-Induced Water Secretion in Rats

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Abstract: Background: Newer antidiarrheal agents include the mucoprotectants gelatin tannate and xyloglucan. Methods: Rat models of cholera toxin (CT)-induced water secretion were used to evaluate the mucoprotective effects of gelatin tannate, xyloglucan, and related compounds. Results: Oral pretreatment for 4 days with gelatin tannate (250 and 500 mg/kg/day), but not tannic acid or gelatin (both 125 mg/kg/day), blocked CT-induced intestinal water secretion. CT-induced intestinal water secretion was also attenuated by oral xyloglucan 12.5 mg/kg + gelatin 125 mg/kg (6 h pre-CT) and gelatin 250 mg/kg (12 h pre-CT), and by local (intra-jejunal loop) administration of gelatin, gelatin tannate and xyloglucan concomitantly with CT. Conclusions: Gelatin tannate and xyloglucan + gelatin attenuated CT-induced intra-loop water secretion in this experimental model, supporting previous evidence that their mechanisms of mucosal protection are closely related to their chemical structures, which confer film-forming properties via the formation of mucoadhesive films.

Keywords: gelatin tannate; xyloglucan; cholera toxin-induced water secretion; animal models of diarrhea; mucoprotectants; mucoadhesive films

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1. Introduction

The small intestine and colon are normally highly effective at absorbing fluid, nutrients and electrolytes from the liquid contents of the upper gut, resulting in the formation of solid stools. However, when components of the gut barrier fail to exert their normal physiological action, pathogens can penetrate the intestinal mucosa and disrupt function [1–3]. Altered bowel homeostasis, due to either active intestinal fluid secretion or impaired fluid absorption, leads to loose and watery stools which are passed several times daily. Diarrhea can be acute (<2 weeks) or chronic [1–3]. The most common cause of diarrhea is infection, which can involve a range of bacterial (*Escherichia coli*, *Shigella* spp., *Vibrio cholerae*), parasitic (e.g., *Giardia* and *Cryptosporidium* spp.) and viral (e.g., noroviruses and rotaviruses) pathogens [1–3].

As dehydration is the primary problem related to diarrhea, rehydration with a balanced oral rehydration solution (ORS) is a major therapeutic measure. Other therapeutic options include antidiarrheal medications and probiotics. Most cases of diarrhea do not require antimicrobials, although such treatment may be appropriate in patients with documented bacterial or parasitic infections [1–3].

Newer agents for the management of diarrhea include mucoprotectants such as gelatin tannate, a complex of gelatin and tannic acid, and xyloglucan, a water-soluble hemicellulose. Xyloglucan is often combined with gelatin to prolong its availability within the intestine [4]. Mucoprotectants act by improving the resistance of the intestinal mucosa to pathogens and restoring normal physiological function [4,5]. Gelatin tannate and xyloglucan ± gelatin are approved as medical devices for the oral treatment of diarrhea but their mechanism of action warrants further investigation [4,5].

Cholera toxin (CT), the main virulence factor of *V. cholera*, indirectly stimulates the cystic fibrosis transmembrane conductance regulator to secrete chloride ions, resulting in the movement of water into the intestinal lumen [6]. CT is commonly used as a secretagogue in animal models to test the ability of compounds to block increased fluid secretion [6]. Using this established model, we investigated the ability of gelatin tannate, its constituents gelatin and tannic acid, xyloglucan and combinations of these compounds to protect against CT-induced intestinal water secretion in anesthetized rats. We also investigated the mechanism of mucoprotection of gelatin tannate and xyloglucan.

2. Results

2.1. Experimental Set 1

2.1.1. Protective Effect of Gelatin Tannate, Tannic Acid and Gelatin

After 2 hours' exposure to CT, the mean weight of water within the intestinal loop was increased by 3- to 4-fold relative to control. Four days of oral pretreatment with gelatin tannate 250 mg/kg/day or 500 mg/kg/day significantly ($p < 0.05$) attenuated mean CT-induced intra-loop fluid secretion relative to vehicle. Pretreatment with tannic acid 125 mg/kg or gelatin 125 mg/kg had no significant effect on mean CT-induced intra-loop fluid secretion relative to vehicle (Figure 1).

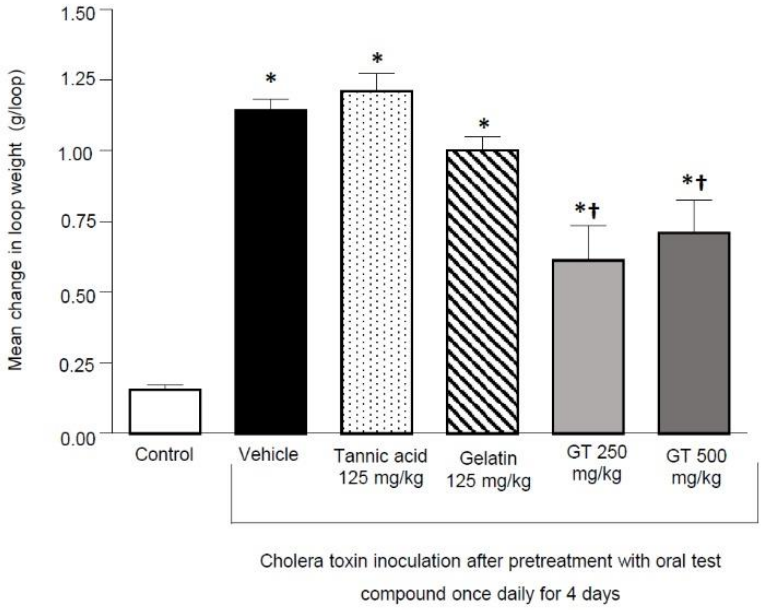

Figure 1. Four days' oral pretreatment with gelatin tannate 250 mg/kg/day or 500 mg/kg/day prior to cholera toxin (CT) inoculation significantly attenuated mean CT-induced water secretion relative to vehicle in isolated jejunal loops of anesthetized rats ($n = 8$/group). * $p < 0.05$ vs. control, † $p < 0.05$ vs. vehicle.

2.1.2. Local Effect of Gelatin Tannate, Tannic Acid and Gelatin

After the 2-h CT challenge, the mean weight of the intra-loop contents was increased by 3- to 4-fold relative to control. Intra-loop administration of gelatin 1.25 mg/loop and gelatin tannate 2.5 mg/loop or 5.0 mg/loop significantly ($p < 0.05$) suppressed mean CT-induced intra-loop water secretion relative to vehicle. In contrast, intra-loop administration of tannic acid 1.25 mg/loop significantly ($p < 0.05$) increased mean CT-induced intra-loop water secretion relative to vehicle (Figure 2).

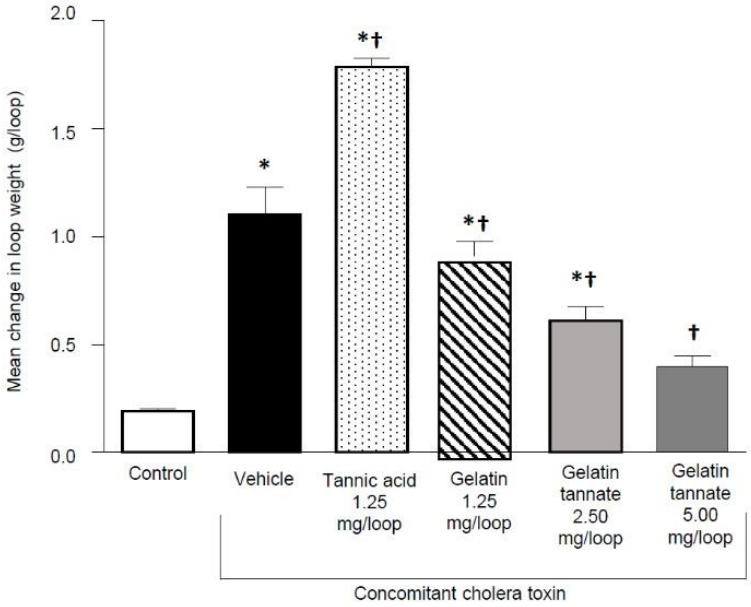

Figure 2. Local (intra-jejunal loop) administration of gelatin 1.25 mg/loop and gelatin tannate 2.5 mg/loop or 5.0 mg/loop significantly attenuated mean cholera toxin-induced water secretion relative to vehicle in isolated jejunal loops of anesthetized rats (n = 8/group). * $p < 0.05$ vs. control, † $p < 0.05$ vs. vehicle.

2.2. Experimental Set 2

2.2.1. Local Effect of Xyloglucan

After 2 h of contact with CT, the mean weight of the intra-loop contents was increased by 60% to 75% relative to control. Intra-loop administration of xyloglucan 0.125 mg/loop significantly ($p < 0.05$) attenuated the CT-induced increase in mean intra-loop water secretion relative to vehicle, whereas xyloglucan 0.075 mg/loop had no significant effect (Figure 3).

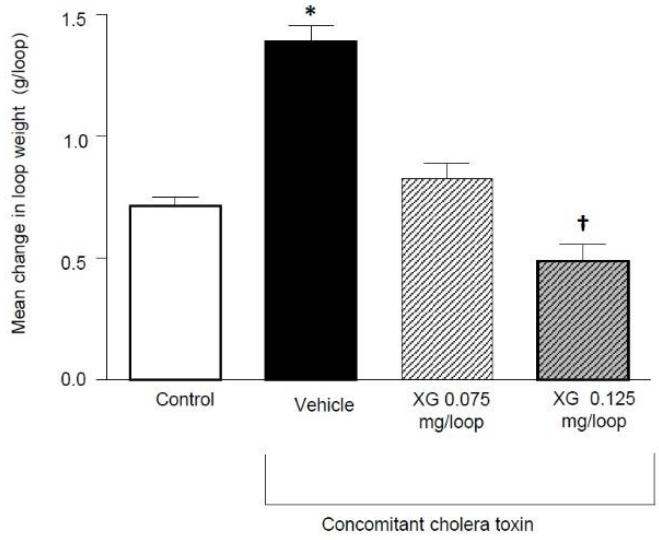

Figure 3. Local (intra-jejunal loop) administration of xyloglucan 0.125 mg/loop significantly attenuated cholera toxin-induced intra-loop water secretion relative to vehicle in isolated jejunal loops of anesthetized rats (n = 8/group). * $p < 0.05$ vs. control, † $p < 0.05$ vs. vehicle.

2.2.2. Duration of Protective Effect with Gelatin Tannate, Xyloglucan, and Gelatin (Alone or in Combination)

After CT challenge for 2 h, the mean weight of the intra-loop contents was increased by 40% to 50% relative to control. Pretreatment with single-dose oral xyloglucan 12.5 mg + gelatin 125 mg/kg 6 h prior to CT challenge significantly ($p < 0.05$) attenuated mean CT-induced intra-loop water secretion relative to vehicle. None of the other oral test compounds or associations had a significant effect on mean intra-loop water secretion (Figure 4).

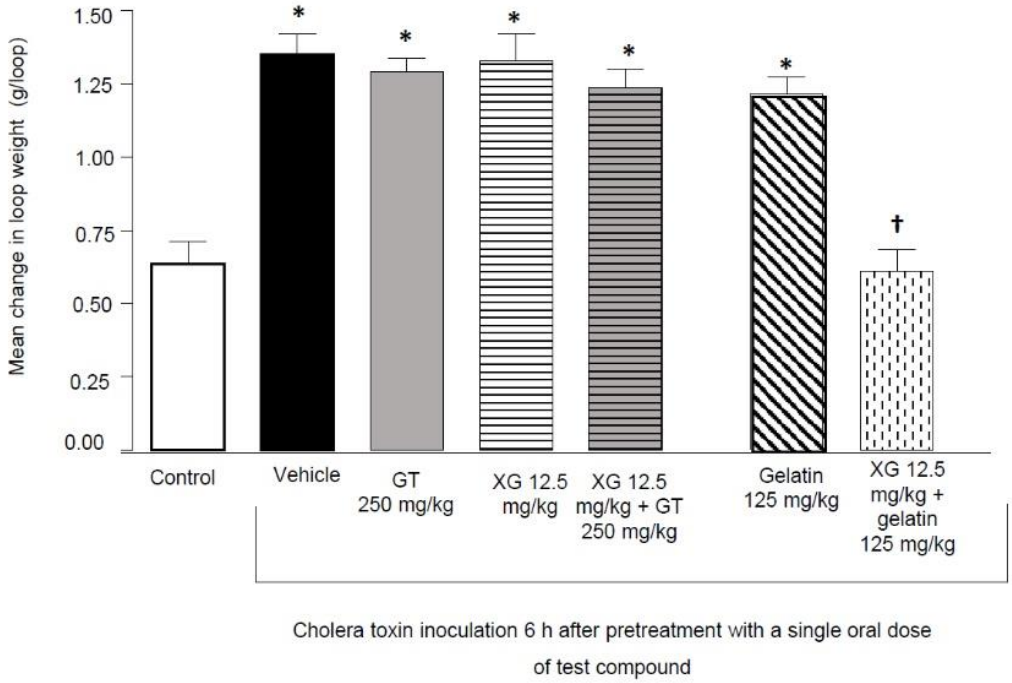

Figure 4. Pretreatment with single-dose oral xyloglucan 12.5 mg + gelatin 125 mg/kg 6 h before cholera toxin (CT) inoculation significantly attenuated CT-induced intra-loop water secretion relative to vehicle in isolated jejunal loops of anesthetized rats ($n = 8$/group). * $p < 0.05$ vs. control, † $p < 0.05$ vs. vehicle.

2.3. Experimental Set 3

Influence of Gelatin Dose on Protective Effect of Xyloglucan + Gelatin

After 2 hours' exposure to CT, the mean weight of the intra-loop contents was increased significantly relative to control. Pretreatment with single-dose xyloglucan 12.5 mg/kg + gelatin 250 mg/kg 12 h before CT challenge significantly ($p < 0.05$) attenuated mean CT-induced intra-loop water secretion relative to vehicle. Xyloglucan 12.5 mg/kg + gelatin 125 mg/kg also reduced the mean CT-induced effect, although the difference relative to vehicle was not statistically significant ($p = 0.07$) (Figure 5).

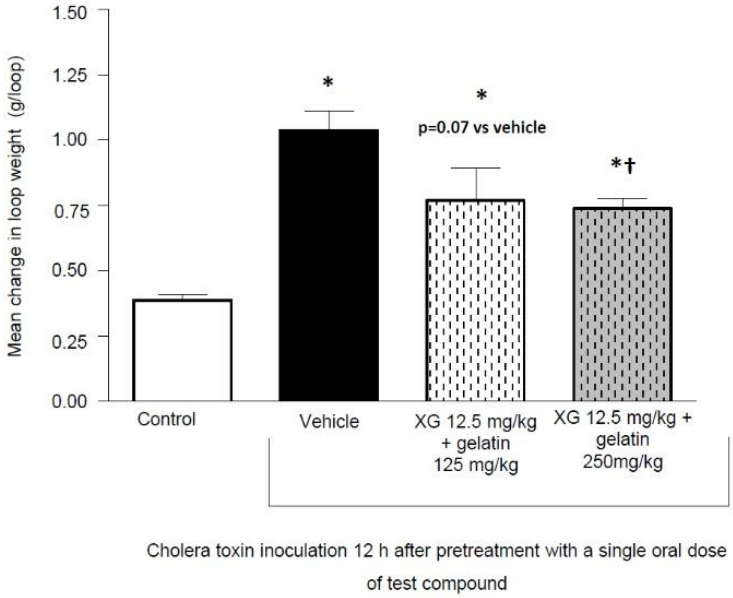

Figure 5. Pretreatment with single-dose oral xyloglucan 12.5 mg/kg + gelatin 250 mg/kg 12 h prior to cholera toxin (CT) inoculation significantly attenuated CT-induced intra-loop water secretion in isolated jejunal loops of anesthetized rats (n = 8/group). * $p < 0.05$ vs. control, † $p < 0.05$ vs. vehicle.

3. Discussion

CT-induced fluid accumulation in isolated jejunal loops of anaesthetized rats is a well-established in vivo animal model used to assess the antisecretory effects of various compounds [6]. Under experimental conditions of repeated and single-dose oral pretreatment with test compound(s) prior to CT inoculation, and local (intra-loop) administration of test compound(s) concomitantly with CT inoculation, we showed that gelatin tannate and xyloglucan + gelatin attenuated CT-induced water secretion in this rat model.

Gelatin tannate, a stable combination of gelatin and tannic acid (penta-m-digallolyl-glucose), traverses unaltered through the stomach. Upon reaching the intestine, it acts in a non-dissociated form as a mucoadhesive film [7] which is generated via electrostatic bonds between gelatin tannate and mucins [8]. Evidence of the mucoadhesive film-forming properties of gelatin tannate derives from an experimental colitis model, in which investigators used confocal microscopy to show the polymeric layer that gelatin tannate forms on ulcerated mucosa [9]. The mechanism of intestinal mucosa protection provided by gelatin tannate is attributed largely to its undissociated form [7]. Our results confirmed this finding using the isolated jejunal loop model. Four days of oral pretreatment with gelatin tannate was effective against the secretory effect of CT at the higher (500 mg/kg) and lower (250 mg/kg) doses administered. Gelatin tannate was still present in the small intestine 4 h after the last administration where it acted locally to block the secretory effects of CT. Neither tannic acid 125 mg/kg nor gelatin 125 mg/kg administered alone for 4 days was able to reduce CT-induced intra-loop water secretion.

To further characterize the mode of action of gelatin tannate (i.e., protection via mucoadhesive film generation), we administered gelatin tannate and its constitutive components locally (i.e., inside the jejunal loop) at the same time as CT inoculation of the loop. Using doses adapted for local administration, gelatin tannate and gelatin reduced the CT-induced effect, whereas tannic acid remained ineffective. The positive effect of locally administered gelatin observed in this experiment may be explained by its polymeric structure which remains unaltered due to the limited solubility of gelatin in short intestinal segments and over the short exposure period. The interplay between gelatin tannate and the mucus network may also contribute to trapping of CT, strongly limiting its access to the intestinal epithelium and consequent water secretion. Local administration of tannic acid not only failed to prevent CT-induced intra-loop fluid secretion, it exacerbated the effect.

A recent in vitro study reported that tannic acid exerts a toxic effect on porcine intestinal cells IPEC-J2 through a mitochondrial pathway of apoptosis and S phase arrest of the cells [10]. The maximal tannic acid concentration applied in cells was 80 µM (1.13 g), which corresponds roughly to the dose of tannic acid (1.25 g/loop) administered in the jejunal loop in our study, likely explaining the strong adverse fluid-secretory effect we observed.

Next, we evaluated the direct local effects of xyloglucan and the duration of its protective effects when administered alone or in association with other test compounds. Xyloglucan is a natural biodegradable high-molecular-weight branched polysaccharide (hemicellulose) derived from the tamarind seed. In the form of an aqueous solution, it possesses high viscosity, broad pH tolerance and adhesiveness, and is not absorbed in the intestine [11]. The configuration of xyloglucan gives it a 'mucin-like' molecular structure, thus conferring optimal mucoadhesive properties [11]. Xyloglucan is commonly used in medical devices for the management of gastrointestinal, urinary and nasal disorders due to its film-forming properties [11]. The rationale for investigating xyloglucan in association with gelatin was based on a previous in vitro experiment. In a co-culture of Caco-Goblet cells™, xyloglucan + gelatin decreased the bacterial adsorption of *E. coli*, particularly in the homogenate mucus compartment [12].

Local administration of xyloglucan 0.125 mg/loop, but not 0.075 mg/loop, at the same time as CT inoculation prevented CT-induced intra-loop fluid secretion, supporting the ability of xyloglucan to exert its mucoprotective effects through the formation of a mucoadhesive film and suggesting that further investigation of higher doses of oral xyloglucan is warranted. Pretreatment with single oral doses of xyloglucan 12.5 mg/kg or gelatin tannate 250 mg/kg, alone and combined, 6 h prior to CT inoculation failed to prevent CT-induced intra-loop water secretion, likely because the film-forming properties of either agent could not occur over this short period. Although xyloglucan 12.5 mg/kg and gelatin 125 mg/kg administered 6 h before CT inoculation were ineffective separately, the combination strongly attenuated CT-induced intra-loop water secretion. Moreover, xyloglucan 12.5 mg/kg + gelatin 250 mg/kg significantly attenuated CT-induced effects even when administered 12 h prior to CT inoculation. The positive effects of xyloglucan and gelatin in combination may be attributed to interactions in the stomach occurring between the secondary hydroxyl groups present in the xyloglucan structure, which confer an anionic charge [13], and gelatin, which has a positive charge at pH < 6 [14]. This enhances the formation of the protective polymeric film, minimizing the contact between CT and the intestinal epithelium.

Taken together, our data, which are based on oral and local administration of several related products, showed that gelatin tannate and xyloglucan + gelatin prevented CT-induced water secretion in isolated jejunal loops in rats, supporting previous evidence that their mechanism of protection involves the ability to form mucoadhesive films.

The film-forming protective properties of gelatin tannate and xyloglucan + gelatin have been exploited successfully in the treatment of acute diarrhea. A randomized trial found that gelatin tannate + ORS was more effective than ORS alone in treating acute diarrhea in children [15]. A systematic review and meta-analysis of clinical studies investigating the antidiarrheal effect of gelatin tannate concluded that it improves stool frequency and stool consistency in children with acute gastroenteritis [16]. In a randomized, open-label study in adults with acute diarrhea, xyloglucan was fast-acting, safe and more effective than diosmectite + *Saccharomyces bouliardii* [17]. A randomized, open-label multicenter study in children with acute gastroenteritis found that repeated xyloglucan + gelatin, in combination with ORS, improved stool consistency on days 3 and 5 of treatment relative to ORS alone [18].

4. Materials and Methods

4.1. Animals

Male Wistar rats (weight 200–225 g) were used in all experiments (8 rats per treatment group). After an overnight fast, rats were anesthetized with subcutaneous pentobarbitone

sodium (60 mg/kg) and anesthesia was maintained by re-administration of pentobarbitone (15 mg/kg/h). The temperature of anesthetized animals was maintained at 35 °C using a heated pad. The trachea was cannulated to prevent airway obstruction. A midline laparotomy was performed to expose the intestine and four 10 cm long jejunal segments were isolated. The lumen was rinsed with hot saline which was followed by CT challenge (replacement with 1 mL sterile NaCl 0.9% containing 10 µg CT). Intestinal segments were resituated in the peritoneal cavity and the abdomen was closed. Two hours later, the animals were sacrificed and each closed intestinal segment was weighed before and after emptying the contents.

4.2. Experimental Design

Three sets of experiments repeated twice were conducted. The first set evaluated the protective properties and mode of action of gelatin tannate, gelatin and tannic acid on CT-induced intra-jejunal loop water secretion. The second set evaluated the direct local effect of xyloglucan, the duration of protection of xyloglucan, gelatin tannate and gelatin and their association on CT-induced intra-jejunal loop water secretion. The third set evaluated the influence of gelatin dose on the duration of protection with xyloglucan.

Doses of test compounds were determined according to previous experiments conducted in our laboratory. Two experimental designs were used: (i) oral pretreatment with test compound(s) before CT inoculation of jejunal loops; and (ii) local (intra-jejunal loop) administration of test compound(s) concomitantly with CT inoculation.

In all experiments, the effects of the test compound(s) in treated groups of rats (test compound + CT in 1 mL NaCl 0.9% in the jejunal segment) were compared with those in the vehicle group (0.25 mL NaCl 0.9% + CT in 1 mL NaCl 0.9% in the jejunal segment). Each experiment also included a control group (0.25 mL NaCl 0.9% + 1 mL NaCl 0.9% in the jejunal segment). The primary endpoint was the mean change in jejunal loop weight (expressed in grams/loop) following exposure to CT for 2 h. This was determined by measuring the weight of jejunal loops in sacrificed rats before and after emptying their contents (i.e., full vs. empty).

4.3. Experimental Set 1

4.3.1. Protective Effect of Gelatin Tannate, Tannic Acid and Gelatin

Five groups of eight rats were pretreated orally once daily for four consecutive days with gelatin tannate 250 or 500 mg/kg, tannic acid 125 mg/kg, gelatin 125 mg/kg or vehicle. The last dose of test compound was administered 4 h before anesthesia and intestinal loop challenge with CT. A control group was pretreated orally with vehicle prior to intra-loop inoculation with sterile saline.

4.3.2. Local Effect of Gelatin Tannate, Tannic Acid and Gelatin

Five groups of eight rats received intra-loop administration of gelatin tannate 2.5 or 5.0 mg/loop, tannic acid 1.25 mg/loop, gelatin 1.25 mg/loop, or vehicle concomitantly with CT inoculation. A control group received intra-loop administration of sterile saline without concomitant CT inoculation.

4.4. Experimental Set 2

4.4.1. Local Effect of Xyloglucan

Three groups of eight rats received intra-loop administration of xyloglucan 0.075 or 0.125 mg/loop or vehicle concomitantly with CT inoculation. A control group received intra-loop administration of sterile saline.

4.4.2. Duration of Protective Effect with Gelatin Tannate, Xyloglucan, and Gelatin (Alone or in Combination)

Six groups of eight rats were pretreated orally with single doses of gelatin tannate 250 mg/kg, xyloglucan 12.5 mg/kg, gelatin 125 mg/kg, xyloglucan 12.5 mg/kg + gelatin

tannate 250 mg/kg, xyloglucan 12.5 mg/kg + gelatin 125 mg/kg or vehicle 6 h before intra-loop administration of CT. A control group was pretreated orally with vehicle 6 h before intra-loop inoculation with sterile saline.

4.5. Experimental Set 3

Influence of Gelatin Dose on the Protective Effect of Xyloglucan + Gelatin

Three groups of eight rats were pretreated orally with xyloglucan 12.5 mg/kg plus gelatin 125 or gelatin 250 mg/kg or vehicle 12 h before intra-loop inoculation with CT. A control group was pretreated orally with vehicle 12 h before intra-loop inoculation with sterile saline.

4.6. Statistical Analysis

The software GraphPad Prism 8.3 (GraphPad, San Diego, CA) was used for statistical analyses. The mean and standard error of the mean (SEM) of the change in intra-loop fluid weight was calculated for each group in each experiment. Comparisons between different treatment arms were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-test. Statistical significance was set at $p < 0.05$.

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