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A natural flavonoid and synthetic analogues protect the gastric mucosa from aspirin-induced erosions

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Abstract

The anti-ulcerogenic properties of plantain banana have been well established even though the active ingredient has only recently been identified as the flavonoid leucocyanidin. The aim of this study was to evaluate the ability of the natural flavonoid leucocyanidin and synthetic analogues to protect the gastric mucosa against aspirin challenge. Natural and synthetic flavonoids were added to the diet of rats, and their anti-ulcerogenic potential evaluated using a prophylactic animal model. Leucocyanidin and its synthetic hydroxyethylated and tetrallyl derivatives were found to protect the gastric mucosa from aspirin-induced erosions. Leucocyanidin and its hydroxyethylated and tetraallyl derivatives significantly increased mucus thickness. Whilst the mechanism by which the natural and synthetic flavonoids protect the gastric mucosa remains to be fully elucidated, it may, as indicated in this study, involve an increase in mucus thickness.

Keywords: Flavonoids; Leucocyanidin; Ulcer; Plantain banana

1. Introduction

The anti-ulcerogenic properties of banana were reported in 1976 by Elliot and Heward [1] and have since been confirmed by others [2,3]. However, until recently, the identity of the active anti-ulcerogenic ingredient has remained elusive. In a recent study, we suggested that the active protective agent of plantain banana is the polyphenolic flavonoid, leucocyanidin [4]. Flavonoids are ubiquitous plant pigments known to have numerous physiological effects that are important for good health [5].

The gastroprotective action of natural flavonoids may be mediated through a stimulation of mucus and bicarbonate secretion [6] or a direct inhibitory effect on the proton pump of the parietal cell [7].

In this study, the ability of the active ingredient derived from plantain banana, leucocyanidin and synthetic leucocyanidin analogues (Aston molecules Ltd., Aston Science Park, Birmingham, UK) to protect the gastric mucosa from aspirin-induced ulceration was evaluated. We also present data that suggests that the protective effect of these flavonoids may be mediated by a stimulation of mucus secretion.

2. Methods and materials

2.1. Animals

Male Wistar rats of average weight (250 g; range: 220–330 g) were used throughout this study.

2.2. Banana samples

The bananas used throughout this investigation were grown in the Varanasi district of India, and were kindly supplied by the late Professor A.K. Sanyal (College of Medical Sciences, Banaras Hindu University, Varanasi, India). The unripe green plantain bananas were peeled and the pulp sun dried and powdered.

2.3. Preparation of leucocyanidin analogues

Purified monomeric leucocyanidin (3,3′,4,4′,5,7-hexahydroxyflavan) was synthesised by the reduction of taxifolin with sodium boron hydride (Aston Molecules Ltd). Purified

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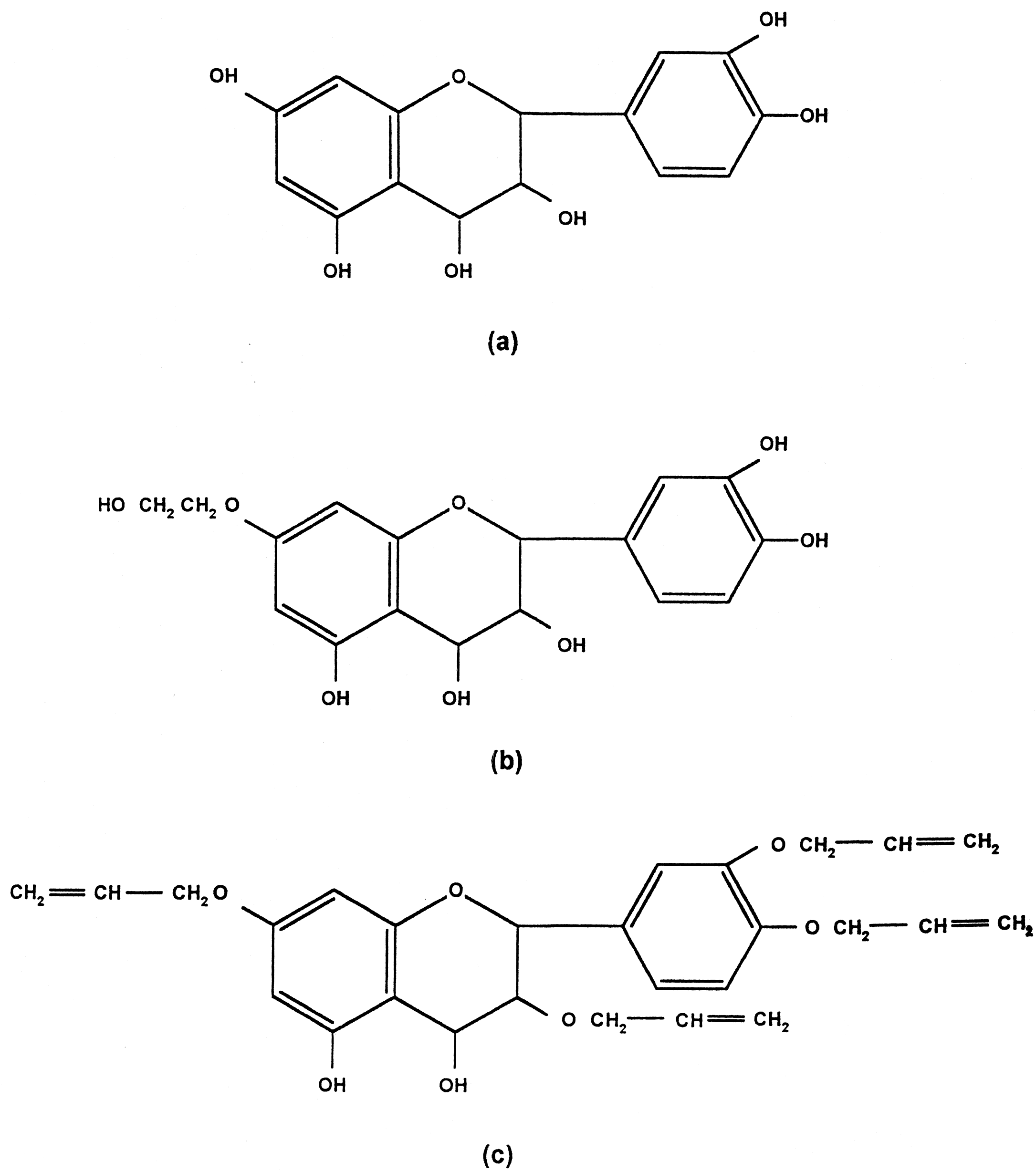

Figure 1. Chemical formula of (a) leucocyanidin (3,3′,4,4′,5,7-hexahydroxyflavan); (b) hydroxyethyl leucocyanidin (3,3′,4,4′,5,7-hexahydroxy-7-hydroxyethylflavan); (c) tetraallyl leucocyanidin (4,5-dihydroxy-3,3′,4′,7-tetraallyloxyflavan).

hydroxyethylated leucocyanidin (3,3′,4,4′,5,7-hexahydroxy-7-hydroxyethylflavan) was synthesised by hydroxyethylation and subsequent reduction of taxifolin with sodium boron hydride (Aston Molecules Ltd). A tetrallyl leucocyanidin derivative (4,5-dihydroxy-3,3′,4′,7-tetraallyloxyflavan) was also provided by Aston Molecules Ltd.

The structure of these compounds was verified by mass spectroscopy, ^{1}H-NMR spectroscopy and ^{13}C-NMR spectroscopy, and are shown in Fig. 1.

2.4. Anti-ulcer activity of flavonoids

The anti-ulcer activity of these flavonoids was assayed using a prophylactic animal model described previously [2]. Animals were allowed free access to water throughout the study.

2.4.1. Prophylactic model

In this procedure, the flavonoids were used to prevent ulceration induced by subsequent aspirin challenge. Individual male Wistar rats were allowed to acclimatise in wire-bottomed cages for 48 h before being fed 14 g of food per day for 48 h. The food was carefully weighed and added to a small amount of water to form a moist paste. Preliminary experiments had shown that this amount of food was completely eaten. The rats were then fasted for 2 days and then challenged with an oral dose of acetyl salicylic acid (aspirin, 150 mg/kg body weight) suspension or vehicle (saline). Five hours later, the animals were killed by ether overdose, the stomachs removed, carefully cut along the greater curvature and washed in ice-cold saline. The ulcer index of each stomach was determined as described previously [2]. Scoring categories contributing to the ulcer index included long linear ulcers in excess of 10 mm (4 points each), medium ulcers less than 10 mm in length (2 points), small circular ulcers less than 1–2 mm diameter (1 point) and small circular ulcers less than 0.25 mm diameter (0.5 points). In addition, the fraction of the stomach showing evidence of haemorrhage (maximum score of 2 points) and the fraction of the stomach showing evidence of transparency to backlighting (maximum score of 2 points) also contributed to the overall ulcer index. In the test group, the flavonoids were added as required to the diet. In control animals, flavonoid was omitted from the diet.

2.5. Determination of mucus thickness

The prophylactic model described previously was employed, and mucus thickness determined 5 h after challenge with either aspirin or vehicle. A modification of the method described by Kerss et al. [8] was used to measure mucus thickness throughout this study. During preparation and handling of the tissue, care was taken not to disturb the mucus layer. Individual animals were fed diets containing 5 mg/day of the appropriate flavonoid. The animals were killed by ether overdose and the stomachs removed. The outer muscle layer was separated from the surface mucosa by injecting a small amount of air between the two layers using a small needle, thereby inducing 'blistering.' A strip of mucosa was dissected out and sections 1.6 mm in length were prepared. These strips were placed on their sides on a specially constructed mount in a petridish containing 0.9% (w/v) saline and viewed using dark-field illumination against background tungsten light and incident ultraviolet light. A minimum of 20 measurements were made of the mucus thickness in each section. At least three sections were taken from each stomach.

2.6. Statistical analysis

Dose-response data are expressed as means ± S.E.M. and significant effects (*$P < .05$, **$P < .01$) of flavonoid treatments were determined using the Wilcoxon rank sum test. Mucus thickness data are expressed as means ± S.E.M. and significant effects of flavonoid treatment were determined using the unpaired Student's t test and a value of $P < .05$ was considered significant.

3. Results

The rat stomach is divided into two distinct regions: glandular and nonglandular (Fig. 2). In the healthy stomach,

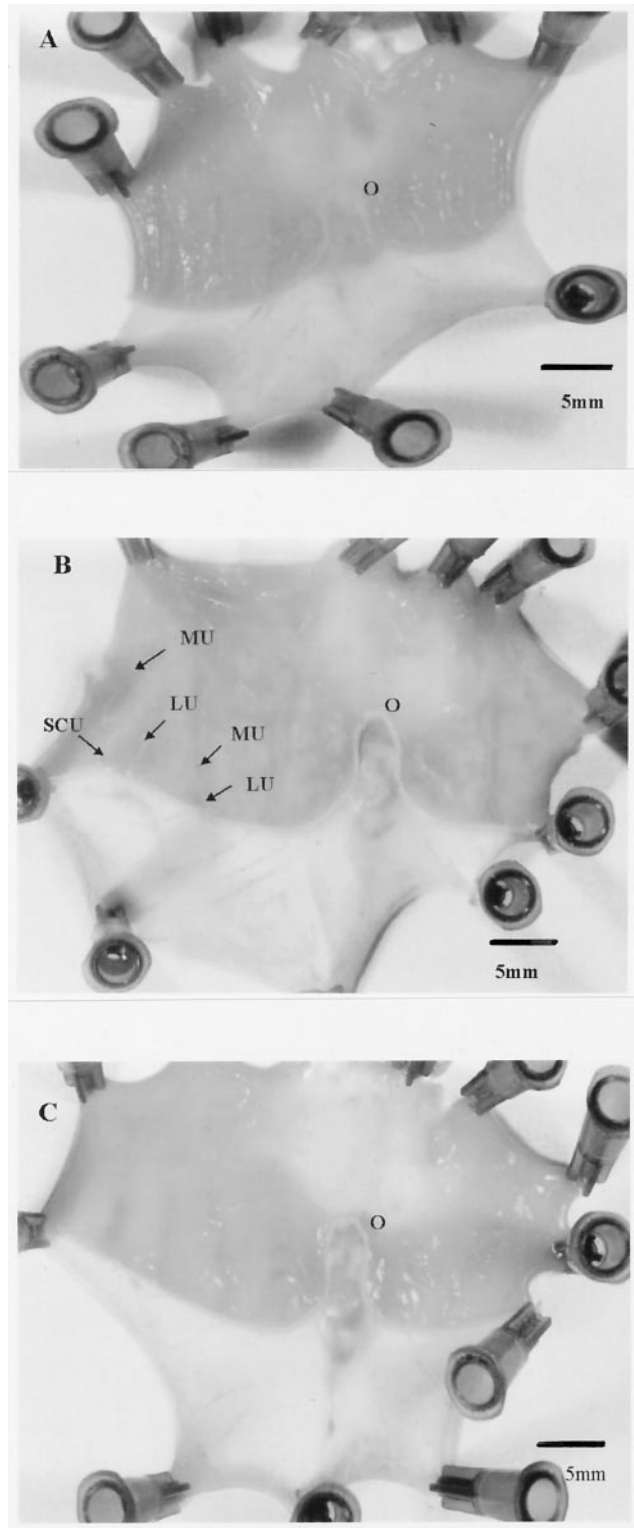

Figure 2. Condition of the rat gastric mucosa following (a) administration of normal diet and saline challenge; (b) administration of normal diet and aspirin challenge (150 mg/kg body weight); (c) administration of leucocyanidin enriched diet and aspirin challenge (150 mg/kg body weight). Key: LU, long ulcers; MU, medium ulcers; SCU: small circular ulcers; O, oesophagus. For further details, see Materials and methods.

the glandular region has a red coloration and is readily distinguished from the nonglandular region.

3.1. Anti-ulcerogenic activity of natural and synthetic flavonoids

Animals challenged with saline consistently scored below 2.5 on the ulcer index. The glandular region of the stomach appears pink and is devoid of ulcerative damage. There is some evidence of transparency to backlighting in an area located centrally in the glandular region. This region represents approximately 10% of the stomach area, and thus contributes 0.2 units to the overall ulcer index (Fig. 2a). In all control animals challenged with aspirin, erosions were found only in the glandular region of the stomach (Fig. 2b). The severity of the erosions in animals fed control diets and challenged with aspirin consistently scored above 20 on the ulcer index used in this study [2]. Selected long ulcers (LU), medium ulcers (MU) and small circular ulcers (SCU) are indicated in Fig. 2b. In addition, evidence of transparency to backlighting was observed over 10% of the stomach area.

The natural flavonoid leucocyanidin was found to protect the gastric mucosa from subsequent aspirin challenge, as shown by the absence of mucosal damage (Fig. 2c) and a significant reduction ($P < .01$) in the ulcer index when added to the diet at 5 mg and 15 mg per day (Fig. 3). A significant protective effect of leucocyanidin was not observed when added to the diet at 2.5 mg per day. The synthetic flavonoids, hydroxyethylated leucocyanidin (Fig. 4) and tetrallyl leucocyanidin (Fig. 5), were also shown to significantly ($P < .01$) protect the gastric mucosa of rats when added to the diet at 5 mg and 15 mg per day.

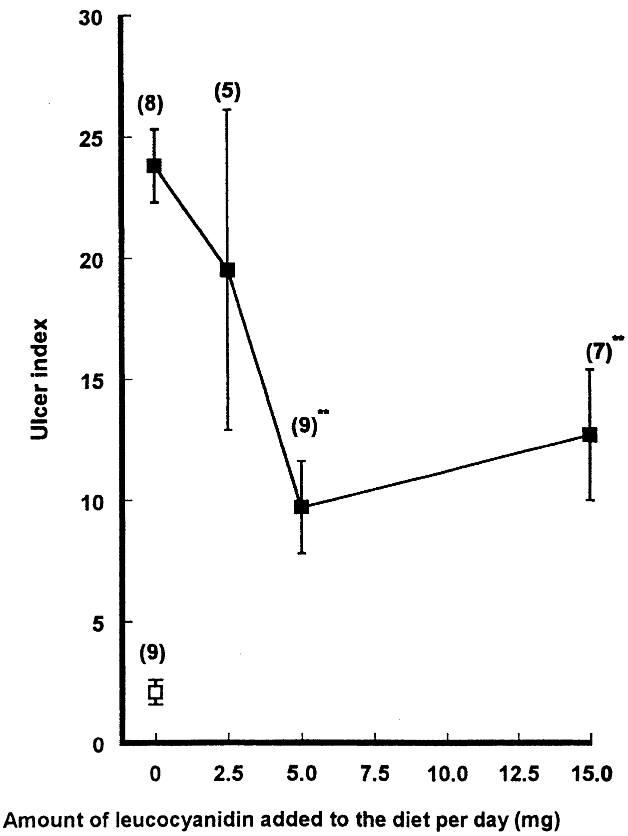

Figure 3. The relationship between the amount of leucocyanidin (mg) added to the diet per day and the ulcer index as determined using the method of Best et al. [2]. The ulcer index values are given as the means ± S.E.M. with the number of repetitions in parentheses. Significant differences between treatments and control diets containing no leucocyanidin were determined using the Wilcoxon rank sum test (**$P < .01$). □, No aspirin challenge.

3.2. Effect of natural and synthetic flavonoids on mucus thickness

The thickness of the mucus barrier protecting the gastric mucosa was determined to be 89 ± 14 μm (13) in control animals fed 14 g of food per day. This was not significantly altered following aspirin challenge. When the diet of animals was supplemented with 5 mg per day of leucocyanidin, hydroxyethylated leucocyanidin or tetraallyl leucocyanidin, the mucus layer significantly increased in thickness to 185 ± 26μm (3), 115 ± 9 μm (3) and 121 ± 11 μm (3), respectively ($P < .02$).

4. Discussion

Although the beneficial effects of banana have been known for some considerable time, we have only recently identified the active anti-ulcerogenic ingredient as the flavonoid leucocyanidin [4]. The present study indicates that oral aspirin challenge (150 mg/kg body weight) causes considerable gastric mucosal damage in the rat (Fig. 2b) and is the first to demonstrate that leucocyanidin (Fig. 2c) and synthetic analogues are protective in this model. Leucocyanidin, hydroxyethylated leucocyanidin and tetrallyl leucocyanidin exhibit significant, though not complete, anti-ulcerogenic potential when added to the diet (20 mg/kg body weight). These observations are consistent with those of other researchers [9] who demonstrated the partial reduction of aspirin-induced gastric lesions in the rat by the synthetic flavonoid, meciadanol. Similar anti-ulcerogenic potential against nonsteroidal anti-inflammatory drugs has also been demonstrated by the natural flavonoid IdB1027 [6].

Banana is known to promote mucus secretion subsequent to stimulation of the growth of mucosal cells, rather than by stimulating mucus secretion directly [2]. In the current study, we demonstrated that leucocyanidin and the synthetic analogues, hydroxyethylated leucocyanidin and tetraallyl leucocyanidin, significantly increase gastric mucus thickness in vivo. However, this is unlikely to be due to a direct stimulatory effect because flavonoids failed to stimulate the release of [^{3}H]-labelled high-molecular-weight material from an isolated gastric cell suspension preincubated with D-[6-^{3}H] glucosamine [10]. Because aspirin is able to penetrate the mucus layer in vivo, it is likely that the increase

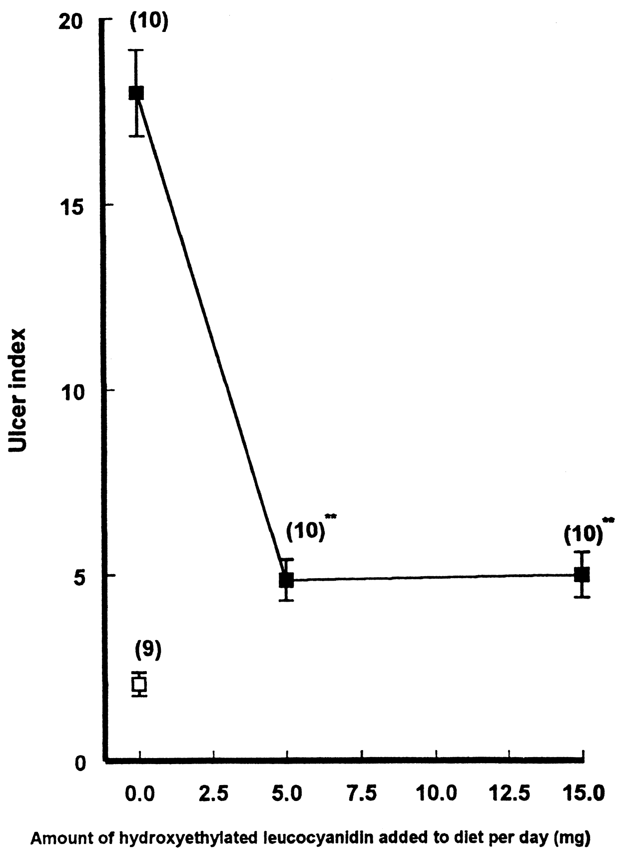

Figure 4. Acute aspirin-induced erosions. The relationship between the amount of hydroxyethylated leucocyanidin (mg) added to the diet per day and the ulcer index as determined using the method of Best et al. [2]. The ulcer index values are given as the means ± S.E.M. with the number of repetitions in parentheses. Significant differences between treatments and control diets containing no hydroxyethylated leucocyanidin were determined using the Wilcoxon rank sum test (**$P < .01$). □, No aspirin challenge.

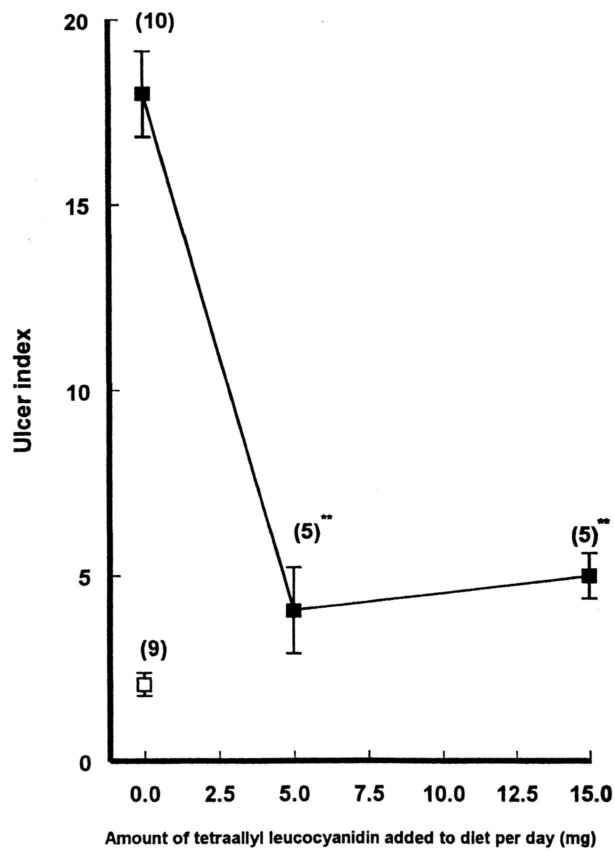

Figure 5. Acute aspirin-induced erosions. The relationship between the amount of tetraallyl leucocyanidin (mg) added to the diet per day and the ulcer index as determined using the method of Best et al. [2]. The ulcer index values are given as the means ± S.E.M. with the number of repetitions in parentheses. Significant differences between treatments and control diets containing no tetraallyl leucocyanidin were determined using the Wilcoxon rank sum test (**$P < .01$). □, No aspirin challenge.

in mucus thickness observed in this study only contributes in part to the protective mechanism of flavonoids in this model. However, this increase in mucus thickness caused by leucocyanidin and synthetic derivatives may still be of importance because it may afford an increased barrier to the back diffusion of hydrogen ions. The effect of both natural and synthetic flavonoids on mucus secretion reported in this study is supported by previous observations that natural flavonoids can reduce, although not prevent, aspirin-induced gastric mucosal damage by increasing mucus and bicarbonate secretion [6].

In conclusion, leucocyanidin and its hydroxyethylated and tetrallyl derivatives protect the gastric mucosa from aspirin challenge. We suggest that the mechanism by which the active agent present in plantain banana protects the mucosa is mediated, at least in part, by an increase in mucus thickness.

Although plantain bananas are extensively grown and eaten as a vegetable in tropical and subtropical countries, the beneficial prophylactic effects described in this study are lost because the bananas are cooked. The banana powder is only active if the fresh pulp is dried at 40°C or lower, as in this study.

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