Br. J. Pharmac. (1984), **82**, 107–116

The anti-ulcerogenic activity of the unripe plantain banana (*Musa* species)

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1 Various preparations of dried unripe plantain banana were found to be anti-ulcerogenic against aspirin-induced ulceration in the rat and were effective both as a prophylactic treatment and in healing ulcers already induced by aspirin. Ripe fruit bananas were inactive.

2 The active factor(s) were water soluble and were concentrated by extraction to approximately three hundred times that in the dried banana powder.

3 The anti-ulcerogenic action of banana preparations appears to be due to their ability to stimulate the growth of gastric mucosa.

4 Aluminium hydroxide, cimetidine, prostaglandin E_2, N^6, O^2-dibutyryl adenosine 3′,5′ cyclic monophosphate but not 5-hydroxytryptamine were also anti-ulcerogenic when used prophylactically in rats but were ineffective in healing ulcers already formed by aspirin. These substances did not stimulate the growth of gastric mucosa.

Introduction

The medicinal properties of the banana are part of the traditions of folk medicine. The plantain banana is collected green and cooked as a food in many third world countries. There are a number of reports (Hanszen, 1934; Elliot & Heward, 1976; Sanyal *et al.*, 1961) which associate the plantain banana with anti-ulcerogenic properties. In this paper we have examined dried unripe plantain banana for anti-ulcerogenic activity in the rat. We have also extracted the active factor and investigated its mode of action and compared it with that of some conventional agents with known anti-ulcerogenic activity.

Methods

Source of banana samples

The bananas used in this work were unripe plantain bananas apart from one sample of ripe fruit banana purchased from a local market. Plantain bananas are also known as vegetable bananas since they have a starchy pulp which requires cooking in order to be edible. Plantain bananas are picked green (unripe) and have a bitter taste when eaten raw. All plantain banana powders used in our studies were from unripe bananas and bitter to the taste. All bananas used were grown solely for commercial reasons and unless otherwise stated it was not possible to specify individual species or varieties. The bananas were from three sources.

(1) Powdered samples of unripe Indian green plantain bananas grown in the Varanasi district were kindly supplied by Professor A.K. Sanyal (College of Medical Sciences, Banaras Hindu University, Varanasi, India). These bananas had been peeled and the pulp sun-dried and powdered. Three such samples were used (Samples A, B and C) which were received in separate batches and varied considerably in their activity and appearance.

Two other samples of plantain banana powder were also supplied by Professor A.K. Sanyal. These bananas were the varieties 'Cavendish' and 'Mondan' also grown in the Varanasi district. These bananas were peeled but dried in ovens at 180°C for 3 min before powdering. Each batch was used separately in the experiments.

(2) Messrs Geest Industries (Peterborough) kindly supplied green plantain bananas from their plantations in St Lucia (West Indies). The bananas were picked 4–5 weeks before ripening. These bananas were peeled and air dried at 50°C before milling (Sample D). Some of these bananas were sliced without peeling before drying and milling (denoted as 'whole banana' or Sample D_1).

(3) We also purchased ripe fruit bananas from a

local Birmingham market, which were peeled, sliced and air dried at 50°C.

The extraction of anti-ulcerogenic factors from banana

Banana powder was subjected to solvent extraction and all extracts and residues were assessed for biological activity against aspirin-induced ulceration by prophylactic or curative procedures. The solvents used in attempts to extract the active factor from banana were water, 95% ethanol, ethanol, acetone, *n*-butanol and chloroform. Organic solvents were evaporated to dryness *in vacuo* using a rotary evaporator and water was removed by freeze drying.

Animals

Male Wistar strain rats weighing 150–200 g were used in these experiments. Seven rats were used in each treatment group.

Induction of ulcers and their scoring (ulcer index)

Ulcers and erosions were induced in the stomachs of fasted rats by acetyl salicylic acid (aspirin, Sigma (London) Ltd., Poole, Dorset). There is some confusion in the literature concerning the difference between an ulcer and an erosion. In this study we have defined an ulcer as a penetration of the glandular mucosa and an erosion as an exfoliation or desquamation of the surface mucosa.

Preliminary experiments established the the oral administration of an aqueous aspirin suspension (150 mg kg^{-1}) (2 ml) to rats previously fasted for 24 h produced a 100% incidence of gastric ulceration. The lesions varied with increasing frequency from deep linear ulcers which were occasionally in excess of 10 mm in length to small circular ulcers up to 2 mm diameter and microscopically (× 20) visible erosions. The edges of the ulcers and erosions were sharply demarcated and frequently the craters were full of blood. It was noted that after aspirin-treatment the stomach wall was frequently fragile and thin. The tissue in the pyloric region and in the lesser curvature appeared transparent when viewed by back-lighting and compared with identical tissue in untreated stomachs. The lesions induced by aspirin were found in the glandular region of the stomach. The severity and extent of gastric damage was scored on a visual basis (ulcer index).

Scoring (ulcer index)

The method of scoring was a modification of the method of Robert & Nezamis (1958), in that we increased the number of categories scored (Table 1). In practise the maximum score found did not exceed 20. A minor modification was made in the scoring system between the prophylactic and curative procedures in that it was noted in the curative procedure that erosions were much less marked. This was probably due to the animals being supplied with food 24 h before they were killed. We therefore substituted a general assessment of the erosive nature of the treatment on the stomach to a maximum score of 2 rather than scoring to a maximum density of 24 erosions per cm^2 as in the prophylactic method. All scoring was carried out by the same observer in all experiments and the identity of the treatments was withheld until scoring was completed. Significance ($P < 0.05$) between treatments was determined by the Wilcoxon rank sum test.

Table 1 The scoring categories used to assess the severity of lesions (ulcer index) induced by aspirin on the rat stomach

Lesion	*Score* *Each*	*Maximum*
Deep linear ulcers > 10 mm length	4	4
Deep linear ulcers < 10 mm length	2	14
Circular ulcers 1–2 mm diameter	1	—
Circular ulcers < 1 mm diameter	0.5	—
Microscopically visible erosions as fraction of 24 cm^{-2}	2	2
Fraction of stomach showing evidence of haemorrhage	2	2
Fraction of stomach showing transparency to back lighting	2	2

Mucosal thickness measurements

After scoring, 1 cm^2 of tissue was removed from the body of the stomach and fixed in Bouin's fluid for 24 h. The tissue was dehydrated in ethanol and cleared for 8 h in chloroform before embedding in paraffin wax blocks. Sections (5 μm) were cut with a Cambridge rotary microtome. The sections were transferred to microscope slides by flotation on 1% w/v albumin solution and after removing excess fluid with adsorbent tissue the sections were left to dry for 12 h at room temperature. The wax was removed from the sections by immersion in xylene and rehydrated by dripping in water for 2 s before staining. The sections were stained with haematoxylin and eosin (HE) and mounted in DPX mounting medium. The maximum and minimum thickness of the gastric mucosa was measured under a microscope (20 ×) and the mean values ± s.e.mean calculated for 10 sections in each treatment. Significance ($P < 0.05$) was determined by Student's *t* test.

Mucosa weights

After scoring the stomachs were frozen and stored at −20°C for 24 h. The samples were allowed to thaw at room temperature. This procedure was found to loosen the surface mucosa from the underlying tissue. The mucosa was removed by scraping with the edge of a microscope slide and freeze dried and weighed. Significance ($P < 0.05$) was determined by Student's t test.

Evaluation of the anti-ulcerogenic action of banana

(1) *Prophylactic method* In this procedure banana was used to prevent or inhibit ulceration induced by aspirin. All animals were housed individually in cages and allowed free access to water throughout the experiments. The animals were allowed to settle in the cages for two days before experiments were started. Weighed amounts of powdered banana were administered either separately or mixed with ground rat pellets (Heygates Ltd., Northampton) as a paste to a total of 14 g each day. This total of 14 g consisted of the weight of banana used made up to 14 g with ground pellets. Preliminary experiments had established that this amount was totally consumed. Each rat was given this diet for two days and then fasted for two days. The aspirin was then administered as an oral aqueous suspension ($150\,mg\,kg^{-1}$). The rats were killed 5 h later and the stomachs removed and washed in ice-cold saline before being examined and scored under a microscope. In the controls banana was omitted from the diet. When soluble anti-ulcerogenic reference compounds or banana extracts were used, they were dosed separately either orally or at the same time as the rats were allowed access to food. The food, when banana extracts or reference compounds were administered separately, consisted of 14 g of ground pellets moistened to a paste.

(2) *Curative method* This procedure was designed to discover whether banana healed ulcers induced by aspirin. Rats were fasted for 2 days and dosed separately with an oral aqueous suspension of aspirin ($150\,mg\,kg^{-1}$). Five hours after dosing the rats were supplied with 14 g of mixed pellets and banana paste. Forty-eight hours after aspirin administration the rats were killed and their stomachs removed and washed and scored as described above.

The anti-ulcerogenic action of cimetidine, prostaglandin E_2 (PGE_2), 5-hydroxytryptamine (5-HT) and N^6, O^2-dibutyryl adenosine 3′,5′ cyclic monophosphate (db cyclic AMP)

Cimetidine was a gift from Smith, Kline & French Laboratories Ltd (Welwyn Garden City, Herts.) and PGE_2 a gift from Upjohn Ltd (Crawley, Sussex). The other materials were purchased from Sigma (London) Ltd., (Poole, Dorset). Both the prophylactic and curative procedures were used. The drugs were administered separately from the food. In the prophylactic procedure the drugs were administered 45 min before aspirin. In the curative procedure the drug was administered in three equal doses at 8 h intervals in the 24 h period when the animals were allowed food. Water was substituted for drugs in the controls. All drugs were administered orally apart from PGE_2 which was given as a subcutaneous (s.c.) injection. The total doses were, cimetidine $240\,mg\,kg^{-1}$; PGE_2 $600\,\mu g\,kg^{-1}$; 5-HT $1.5\,mg\,kg^{-1}$ and db cyclic AMP $600\,\mu g\,kg^{-1}$. The ulcer index values were determined.

The anti-ulcerogenic action of banana compared with the action of aluminium hydroxide

The prophylactic procedure was used. Aluminium hydroxide (BDH Ltd., Poole) was administered as a suspension orally in three doses (each $8\,mg\,kg^{-1}$) during the fasting period. In the antacid control group, aspirin treatment was omitted. Banana sample A (India) (7 g) was used in this experiment.

The anti-secretory activity of banana, cimetidine, PGE_2, 5-HT and db cyclic AMP

The procedure for measuring gastric acid secretion was based on the Shay rat (Shay *et al.*, 1945). After a 2 day fasting period the rats were dosed with the drugs. The amounts used were the same as those used in the anti-ulcerogenic studies described above. Controls were dosed with water, $5\,ml\,kg^{-1}$. Forty-five minutes later the animals were dosed orally with aspirin ($150\,mg\,kg^{-1}$). After a further 45 min the animals were anaesthetized with sodium pentobarbitone and the pyloric sphincter ligated. The rats were maintained under anaesthesia for 5 h before they were killed and the stomachs removed and the gastric juice collected. The volume and the acidity of the juice was determined by titration using phenolphthalein as indicator.

Biochemical studies on gastric mucosa

Hexosamine and sialic acid determinations were carried out as a measure of mucus formation (Lukie & Forstner, 1972) and DNA as a measure of cell proliferation.

Hexosamine determinations

Accurately weighed portions of mucosa (30–40 mg) were digested with 5 ml of 2N HCl in stoppered test

tubes in a boiling waterbath. After cooling and filtration, filtrates were made up to 6 ml with water. Hexosamine determinations were carried out on portions of the extracts by the method of Dische & Borenfreund (1950).

Sialic acid determinations

Weighed portions of the mucosa were treated with 2 ml of 0.1N H_2SO_4 in stoppered test tubes in a waterbath maintained at 90°C for 1 h. Sialic acid levels were determined in portions of the filtrate by the method of Warren (1959).

DNA determinations

The mucosa samples were weighed and dispersed as a homogenate in 10 ml of water. The free DNA was removed by the addition of 0.3 ml of 30% w/v bovine serum albumin followed by vigorous shaking and the addition of 3 ml of 50% w/v trichloracetic acid (TCA). The samples were frozen at − 30°C for 3 days and then allowed to thaw at 37°. The precipitated material was removed by centrifuging at 600 *g* at 4°C. Ten ml of 5% w/v TCA was added to the supernatants and after shaking the samples were centrifuged at 1000 *g* at 4°C for 30 min. The supernatants were carefully removed and discarded. The pellets were hydrolysed with 6 ml of 5% TCA w/v at 90 ± 1°C for 15 min and after cooling the hydrolysates were centrifuged at 1000 *g* for 30 min. The supernatants were retained and the pellets hydrolysed again with 5% w/v TCA as previously described. The second supernatant was combined with the first and the pooled samples frozen at − 30°C and then allowed to thaw. Any precipitate that formed was removed by centrifugation. The supernatants were evaporated to dryness *in vacuo* and the residues dissolved in 4 ml of water. As standards 3,6,9,12,15,18 and 21 μg of highly polymerized calf thymus DNA (Sigma (London) Ltd., Poole, Dorset), were extracted and hydrolysed by the same procedure. The standards were selected to cover the DNA concentrations expected in mucosa samples. Finally the amount of DNA was determined by the colorimetric method of Croft & Lubran (1965). The results were expressed as μg DNA mg^{-1} tissue and as the total DNA in the mucosa from each stomach.

Results

The anti-ulcerogenic activity of banana

(1) *Prophylactic treatment* In this experiment the animals each consumed 5 g of powdered Indian plantain banana (Sample A) before fasting and aspirin treatment. When the banana was omitted an ulcer index of 16.0 ± 1.0 was obtained. When the banana was included in the diet the value dropped to 6.0 ± 1.0 ($P < 0.001$) and rats fasted after a normal diet but not given aspirin scored 2.0 ± 0.5. Clearly this banana preparation was effective in reducing the severity of ulceration.

(2) *Curative method* Seven grams of banana was given to the rats over 2 days following aspirin treatment. Two separate samples of Indian plantain banana were used and ulcer index values were Sample A, 7.8 ± 0.6 ($P < 0.001$) and Sample B, 8.6 ± 0.8 ($P < 0.01$) compared to aspirin-treated controls on a normal diet (ulcer index 16.0 ± 1.0). The mucosa weights were determined in this experiment and were Sample A, 71.0 ± 2.0 mg per rat, ($P < 0.001$), Sample B, 67.5 ± 1.5 mg ($P < 0.01$) compared with 58.8 ± 2.3 mg for aspirin-treated controls. This result indicated that the banana healed the ulcers and that it may have done so by stimulating the growth of the gastric mucosa. The experiment was repeated in order to discover whether Sample D (derived from St Lucia, see methods) was also active. In this experiment the aspirin-treated controls gave an ulcer index value of 15.8 ± 0.8 compared with Sample C (Indian plantain banana) where a value of 8.1 ± 1.8 ($P < 0.001$) was obtained. The St Lucia sample D gave a value of 10.6 ± 1.8 ($P < 0.01$) and the 'whole banana' sample D_1 gave virtually the same value, 10.7 ± 1.2 ($P < 0.01$). The corresponding mucosa weights were for aspirin-treated controls, 61.7 ± 2.6 mg, Sample C, 72.3 ± 2.0 mg ($P < 0.01$), Sample D, 70.0 ± 2.1 mg ($P < 0.02$) and Sample D_1 70.0 ± 1.9 mg ($P < 0.02$). Therefore both the Indian and St Lucia samples were active.

In a further experiment using the curative procedure diets were administered using 2, 3 and 4 g of the Indian plantain banana (Sample A). The results obtained showed a dose-response relationship in that the aspirin controls gave an ulcer index value of 16.6 ± 1.1, compared with banana Sample A, 2 g, 11.0 ± 3.5 ($P < 0.02$), 3 g, 4.7 ± 0.6 ($P < 0.001$) and 4 g, 4.7 ± 0.6 ($P < 0.001$). The corresponding mucosa weights were controls, 56.7 ± 1.4 mg, banana Sample A, 2 g, 59.0 ± 2.9 mg (NS), 3 g, 69.3 ± 1.7 mg ($P < 0.01$) and 4 g, 69.0 ± 2.0 mg ($P < 0.01$). The optimum effective dose of banana was 3 g over the 2 days of diet since banana (4 g) maintained but did not increase the dose effect.

Negative results were obtained when plantain banana samples 'Cavendish' and 'Mondan' (see Methods for sources) were used in the experiments. For these two varieties the total diet was increased from 14 g to 16 g, consisting of 9 g of normal food and 7 g of banana. This was because slightly larger rats were used in this experiment (225 g body weight).

For comparative purposes the Indian plantain banana Sample B was used. The ulcer index values were aspirin-treated controls, 16.8 ± 0.5, Sample B, 8.3 ± 1.3 ($P < 0.01$), 'Cavendish', 16.9 ± 0.5 (NS), 'Mondan' 14.7 ± 1.0 (NS). Mucosa weights were aspirin-treated controls, 56.4 ± 2.8 mg, Sample B, 69.0 ± 1.6 mg ($P < 0.02$), 'Cavendish' 56.3 ± 2.4 mg (NS), 'Mondan' 59.3 ± 2.2 mg (NS). This negative result may have been due to the fact that the bananas had been air dried at 180°C. The fruit banana was also unsuccessful in the test system. When 14 g of dried fuit banana was mixed with 2 g of pellets and compared with a group of rats on a diet of 3 g of Indian plantain banana, Sample A, mixed with 13 g of pellets, the ulcer index values obtained were, aspirin-treated controls, 14.1 ± 0.7, Sample A, 7.0 ± 0.5 ($P < 0.01$), fruit banana 12.4 ± 2.0 (NS).

Extraction of the active anti-ulcerogenic factors from banana

Two solvents, water and 95% ethanol, were found to extract the anti-ulcerogenic factors from powdered banana whereas organic solvents, including anhydrous ethanol, failed to remove the active substances from the powder. When water and aqueous ethanol were used as solvents no residual anti-ulcerogenic activity was left in the powder. Successful extractions were carried out at room temperature as it was found that elevated temperatures destroyed the anti-ulcerogenic activity. The organic solvents removed non-active substances from the banana and were therefore useful for removing unwanted material from the active extracts. Aqueous ethanol was particularly useful as it completely extracted the active substances from the banana powder but did not take into solution various sugars which seriously contaminated the water extractions. After some preliminary trials the extraction scheme in Figure 1 was used to concentrate the active materials in banana.

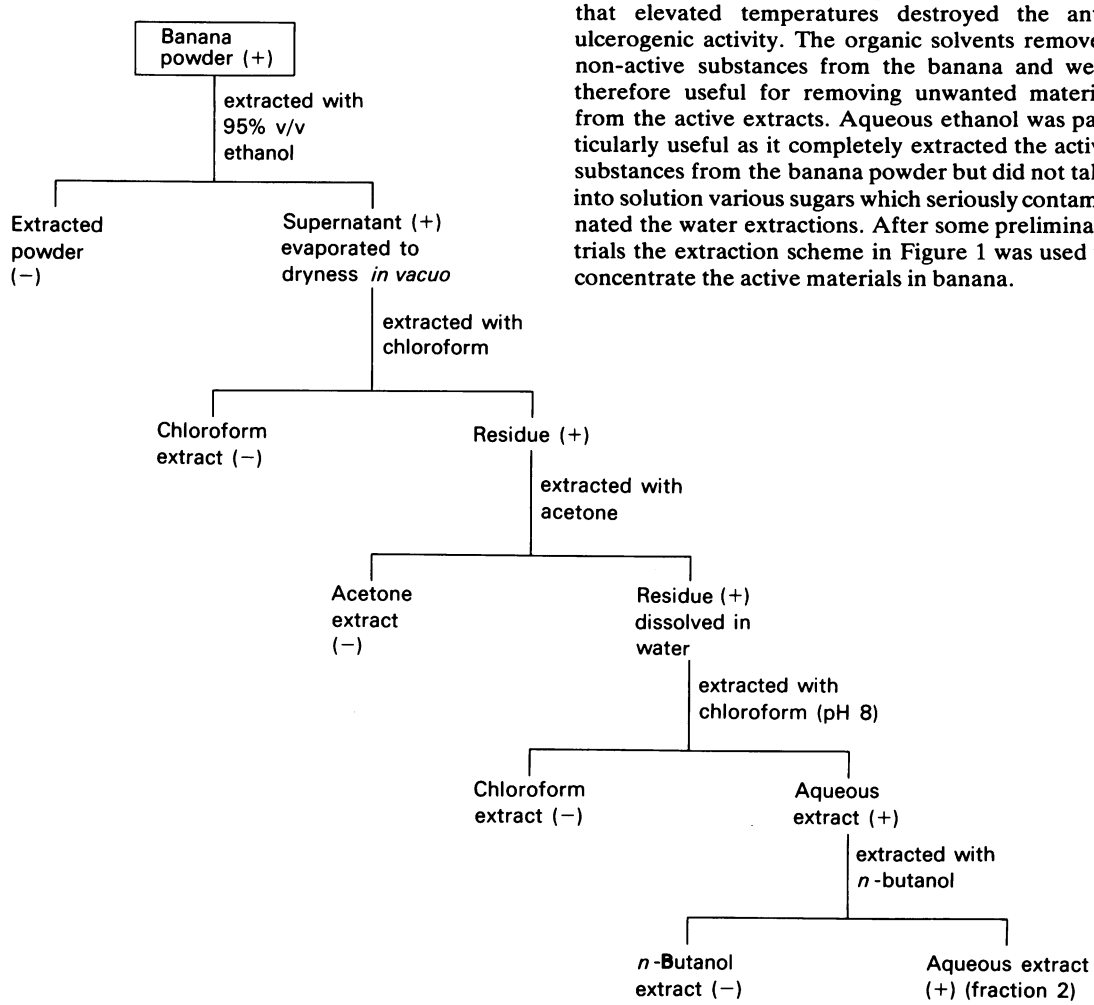

Figure 1 Schemes for the extraction of the anti-ulcerogenic factors from banana powder. All operations were carried out at room temperature. The factors were also extractable by water (fraction 1) but water extraction alone produced problems in further extraction due to a high carbohydrate content. Biological activity: (+) present; (−) absent.

Table 2 The ulcer indices and mucosa weights produced in rats by the final fraction obtained by solvent extraction of banana Sample A

Expt. no.	*Treatment*	*Ulcer index*	*Mucosa weight* (mg)
1	Aspirin-treated controls	15.0 ± 1.3	73.0 ± 2.6
1	Aspirin-treated with 7 g banana powder	10.8 ± 1.2*	79.2 ± 1.8
1	Final aqueous extract (22.5 mg dry wt)	10.0 ± 1.0*	81.7 ± 1.9*
2	Aspirin-treated controls	15.9 ± 0.7	61.5 ± 1.9
2	Final aqueous extract (fraction 2) administered intraperitonedly 22.5 mg	9.8 ± 1.2*	70.5 ± 2.4**

The final aqueous fraction (2) represents the extract from 7 g of banana-powder (dry wt 22.5 mg). The curative method of evaluation was used.
Significance of differences with respect to aspirin-treated controls: * $P < 0.01$, ** $P < 0.02$.

The amounts of active material in the final active aqueous fraction varied with the banana sample used and represented between 0.3–0.7% w/w of the original banana powder. Of the solvents used, 95% ethanol removed polysaccharides such as starch, and protein. Chloroform removed fats, waxes and high molecular weight fatty acids and butanol removed monosaccharides and disaccharides. The total biological activity was retained in the final aqueous fraction (2) as shown in Figure 1. The most active sample was the Indian Sample A. Using Sample A the final aqueous fraction was 0.32% w/w of the original powder and thus this represents a 320 fold increase in concentration of the active material.

In one experiment an active aqueous extract (fraction 2) derived from Sample A was administered by the intraperitoneal route to rats in an experiment using the curative procedure. The results in Table 2 show that the anti-ulcerogenic factors were active by this route as well as by oral administration which suggests an indirect effect by the active factors on the gastric mucosa. An experiment where the aqueous extract of banana was digested with 1% pepsin in 0.2 N HCl before being used in the curative procedure also produced an anti-ulcerogenic effect The pepsin resistance therefore suggests that the anti-ulcerogenic factor(s) is not a protein or peptide.

A comparison of the anti-ulcerogenic activity of banana (Sample A) with that of Al (OH_3)

The following ulcer index values were obtained for various prophylactic treatments: aspirin treatment alone, 17.2 ± 0.8, banana Sample A with aspirin, 7.3 ± 1.8 ($P < 0.005$) and $Al(OH)_3$ with aspirin, 12.4 ± 0.2 ($P < 0.05$). Thus both banana and aluminium hydroxide treatments were anti-ulcerogenic although at the doses used banana treatment was more effective. It was during this experiment that it was observed that differences in mucosa thickness were evident in sections cut from the stomachs. A separate experiment was carried out using the three treatment groups above but also including groups that were treated with banana alone and controls receiving solely rat pellet diet. These latter controls were used to establish a baseline for differences in mucosa thickness as a result of various treatments. With banana treatment alone the thick-

Table 3 The anti-ulcerogenic activity of cimetidine, prostaglandin E_2 (PGE_2), 5-hydroxytryptamine (5-HT) and db cyclic AMP

Treatment		*Ulcer index*	*Mucosa weight* (mg)
None (normal rats)		1.8 ± 0.6	82.3 ± 3.1
Aspirin-treated control		16.1 ± 1.2	81.5 ± 3.1
Cimetidine	prophylactic	7.1 ± 1.2**	80.2 ± 3.7
	curative	14.2 ± 0.8	80.6 ± 1.5
PGE_2	prophylactic	4.4 ± 0.9**	83.0 ± 4.3
	curative	14.3 ± 1.3	85.2 ± 2.5
5-HT	prophylactic	14.0 ± 1.4	81.3 ± 3.2
	curative	13.4 ± 1.7	83.5 ± 3.8
db cyclic AMP	prophylactic	9.4 ± 1.7*	84.8 ± 3.0
	curative	13.0 ± 0.6	85.2 ± 1.9

Signifiance of difference with respect to aspirin-treated controls is shown as follows: ** $P < 0.001$; * $P < 0.01$.
The doses of drugs used are given in Methods.

Table 4 The anti-secretory activity of banana extract, cimetidine, prostaglandin E_2 (PGE_2), 5-hydroxytryptamine (5-HT) and db cyclic AMP

Expt. no.	*Treatment*	*Volume of gastric juice* (ml $100g^{-1}$ weight)	*mEq H^+ per l*	Acid output as μEq H^+ per 5 h
1	Aspirin control	1.5 ± 0.2	35.2 ± 6.3	46.5 ± 6.2
1	Cimetidine	0.3 ± 0.1***	46.0 ± 7.8	12.8 ± 5.2***
1	PGE_2	0.3 ± 0.1***	41.1 ± 8.0	11.0 ± 5.2***
1	5-HT	0.7 ± 0.1*	93.3 ± 12.6*	52.5 ± 7.7
1	db cyclic AMP	1.4 ± 0.2	42.2 ± 6.8	49.3 ± 7.6
2	Aspirin control.	1.1 ± 0.1	80.6 ± 8.7	88.5 ± 7.5
2	Aqueous extract of 7 g banana Sample A (22.5 mg)	1.1 ± 0.1	83.6 ± 8.8	89.2 ± 8.0

Significance of differences with respect to aspirin treated controls is shown as follows: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.
The doses of drugs used are given in Methods.

ness of the glandular mucosa increased by 6.8 μm ($P < 0.02$). Aspirin-treatment decreased the thickness by 7.7 μm ($P < 0.02$) but banana with aspirin prevented this and increased the thickness by 1.4 μm. Aluminium hydroxide treatment alone decreased the thickness by 1.1 μm and in combination with aspirin reduced it by 2.0 μm. For example, with aspirin-treatment alone the average mucosa thickness was 46.7 ± 3.1 μm whereas when the aqueous extract of 7 g banana Sample A was administered with aspirin the average thickness increased to 56.9 ± 2.3 μm ($P < 0.01$). The results above suggest that banana-treatment reduces the erosive effects of aspirin in mucosa. The $Al(OH)_3$ treatment also protected mucosa from aspirin but the effect was less marked than with banana treatment. When banana was given to untreated rats it appeared to promote an increase in thickness of mucosa. This may have been due to a stimulating effect by banana on mucosa cell formation.

The anti-ulcerogenic properties of cimetidine, PGE_2, 5-HT and db cyclic AMP

The values obtained are given in Table 3. There are several points of interest in this table. As expected cimetidine, PGE_2 and db cyclic AMP were anti-ulcerogenic but only as prophylactic agents. In contrast, as shown previously, banana was effective both as a prophylactic and curative agent. Also banana, as reported above, increased the mucosa mass whereas the other substances in Table 3 did not. Therefore, the anti-ulcerogenic action of banana is different from that of the substances used in Table 3 although it is possible that endogenous substances such as cyclic AMP and prostaglandins may be involved in the mechanism of action of banana. 5-Hydroxytryptamine did not protect against aspirin-induced gastric ulceration.

The gastric anti-secretory activity of banana, cimetidine, PGE_2, 5-HT and db cyclic AMP

The anti-secretory action of banana was compared with that of the reference substances shown in Table 4. Banana (Sample A) and db cyclic AMP were not anti-secretory but cimetidine and PGE_2 were very effective in reducing both the volume and acidity of the gastric secretions. Treatment with 5-HT reduced the volume of gastric juice but not its acidity which increased. Hashizume *et al.* (1978) found that 5-HT 20 mg kg^{-1} decreased the volume of gastric juice and the acid output in the Shay rat and induced the formation of gastric erosions. They also found that 5-HT reduced the acidity of the juice. In our experiments we used anaesthetized animals and a lower dose of 5-HT, but whether these experimental differences were responsible for the increase of acidity found in the gastric juice in our experiments instead of a decrease as observed by Hashizume *et al.* (1978) requires further investigation. However, the unripe plantain banana did not inhibit gastric acid secretion.

Sialic acid and hexosamine levels in gastric mucosa

The results for the analysis of mucosa samples taken from rats treated with aspirin only and aspirin in combination with various doses of banana powder (Sample A) are given in Table 5. No significant differences were found in the values when the amounts were calculated in terms of unit weights of the tissue. However, when the total content of sialic acid and hexosamine was calculated, there were significant increases in the amounts in proportion to the

Table 5 Sialic acid and hexosamine levels in gastric mucosa of rats treated with banana preparations

Treatment	*Sialic acid content* ($\mu g\,mg^{-1}$)	(total μg)	*Hexosamine content* ($\mu g\,mg^{-1}$)	(total μg)
Aspirin	0.2±0.0	12.3±1.8	11.4±1.2	651±55
Aspirin + 2 g banana Sample A	0.2±0.0	13.9±1.8	12.4±1.7	725±78
Aspirin + 3 g banana Sample A	0.2±0.0	16.9±1.1*	14.1±1.4	927±96**
Aspirin + 4 g banana Aspirin Sample A	0.3±0.0	18.2±1.6**	13.9±1.4	1031±118***

Significance of differences with respect to aspirin-treated controls: *** $P<0.01$; ** $P<0.02$; * $P<0.05$.

dose of banana used (Table 5). These higher levels correlate with the increased mass of mucosa in the stomachs of animals treated with banana (Table 2).

DNA levels in mucosa

The results in Table 6 show that banana treatment (Sample A) raised DNA levels in both a unit mass of tissue and also in the total mucosa. This result, coupled with the observation that banana treatment increases the mucosa mass, suggests that banana has a stimulating effect on cell proliferation in mucosa.

Discussion

The results show that various preparations of dried powdered unripe plantain banana possess anti-ulcerogenic activity against aspirin-induced gastric ulceration in the rat. Banana possesses activity in inhibiting the induction of ulcers by aspirin and it is also effective in healing these ulcers once formed. Its mechanism of action appears to be by stimulating the growth of gastric mucosa. This stimulatory effect was also found in normal rats and the increased mass of mucosa and the increased production of mucus was probably responsible for the protective effect against aspirin. The stimulatory effect of banana on mucosa growth was probably also responsible for the rapid healing of ulcers in rats treated with banana after aspirin administration. The regenerated mucosa cells would rapidly seal damaged areas with a secretory layer of mucus and prevent further erosions due to gastric HCl and pepsin.

Modern studies on the anti-ulcerogenic activity of banana were stimulated by the discovery that the ripe fruit banana contains high levels of 5-HT (Waalkes *et al.*, 1958; West, 1958). This was confirmed by Sanyal *et al.* (1961) who, noting that 5-HT may be concerned in peristaltic reflexes (Bülbring & Lin, 1956) and in the inhibition of gastric secretion (Black *et al.*, 1956), showed that banana emulsions (varieties not specified) introduced directly into the stomach reduced gastric secretion and ulceration induced by repeated injections of histamine. Sanyal *et al.* (1963) noted that sun-dried plantain banana pulp was a rich source of 5-HT and showed that this material administered orally at $1\,g\,kg^{-1}$ daily to guinea-pigs had a prophylactic effect against phenylbutazone-induced gastric ulceration. The dried pulp was also effective when used prophylactically in guinea-pigs in reducing the incidence of restraint ulcers induced by the

Table 6 The effect of treatment with banana preparations on the DNA levels in gastric mucosa

Treatment	Ulcer *index*	*Mucose weight* (mg)	*DNA* ($\mu g\,mg^{-1}$ tissue)	*Total DNA in mucosa* (μg)
No treatment	1.8±0.2**	73.2±2.1	7.0±1.1	511±78
Aspirin	14.4±0.6	71.2±2.1	7.1±0.7	507±53
Aspirin with banana 3 g (Sample A)	7.0±0.5**	84.9±1.6**	9.6±0.7*	811±59*

Significance of differences with respect to aspirin-treated controls: ** $P<0.001$; * $P<0.02$.

method of Brodie & Hanson (1960) but did not inhibit prednisolone-induced ulceration in guinea-pigs (Sanyal *et al.*, 1963). Our experiments show that 5-HT cannot be the active anti-ulcerogenic principle of plantain banana extracts as it was removed by solvent extraction from the aqueous banana extracts (this was confirmed by t.l.c.). Moreover, we found no evidence that our banana preparations were anti-secretory (Table 4) although they were anti-ulcerogenic. Indeed, other workers have shown that 5-HT at high doses is ulcerogenic in rats (Haverbach & Bogdanski, 1957). Sanyal *et al.* (1963) suggested that the anti-ulcerogenic properties of banana may be due to a demulcent or antacid effect similar to that of $Al(OH)_3$ but we have found no evidence to support this suggestion. Of interest is a paper by Elliot & Heward (1976) who found that the anti-ulcerogenic action of raw ripe fruit banana or histamine-induced gastric ulcers in mice was associated with a gain in fresh stomach weight. We found that ripe fruit banana was inactive but this could be because we dried our banana at 50°C before use.

Musa is a large genus complicated for the purposes of identification by cross hybridisation between edible and wild species. Also the biological activity of banana may vary with the time of harvesting and method of drying. Our own experience supports this. The Indian Samples varied in activity and appearance in that Sample A was more active than Samples B and C. In order to standardise harvesting conditions we obtained a supply from the West Indies (Sample D) which although not as active as the Indian Sample A was dried under standard conditions. Unfortunately the four plantations on St Lucia supplying this banana were destroyed by a hurricane. Two further Indian samples 'Cavendish' and 'Mondan' were found to be inactive but we were informed that these samples had been oven dried at 180°C which probably accounted for their lack of activity. Our banana samples were unripe since plantain bananas lose their activity on ripening (Sanyal, personal communication). We have not tested this experimentally but we have shown that low temperature-dried unripe plantain bananas are anti-ulcerogenic against aspirin-induced gastric ulceration.

The active factors in banana are thermolabile and water soluble but insoluble in organic solvents. The factors are very active since aqueous extracts containing 22.5 mg of material extracted from 7 g of banana powder (Sample A) were active against the aspirin model.

Banana had both a prophylactic and curative effect on aspirin-induced ulcers but cimetidine, PGE_2, and db cyclic AMP only acted prophylactically against the aspirin model. Banana was not anti-secretory but PGE_2, cimetidine and 5-HT were. Banana increased the mucosa weight but cimetidine, PGE_2, 5-HT and db cyclic AMP did not. Consequently it is unlikely that the mechanism of the anti-ulcerogenic action of banana was similar to that of our reference drugs. The mode of action of the banana factor appears to be unlike that of convential anti-ulcerogenic drugs in that it promotes mucus secretion subsequent to stimulation of the growth of mucosa cells rather than by directly stimulating the cells to secrete mucus. Two drugs of natural origin, carbenoxolone (a derivative of glycyrrhizic acid found in liquorice) and gefarnate (present in white headed cabbages) have related actions in that they have been reported to stimulate mucus secretion (Hausmann & Tarnoky, 1966; Takagi & Okabe, 1968). In a comparative trial between carbenoxolone and gefarnate, carbenoxolone was found to be superior in its anti-ulcerogenic properties to gefarnate (Langman *et al.*, 1973). However, carbenoxolone has aldosterone-like side-effects such as hypertension, fluid retention and hypokalemia which limits its clinical usefulness. Since the plantain banana is a bulk constituent of the diet of many third world countries it is unlikely that the anti-ulcerogenic factor present is toxic. The role of banana in folk medicine as an anti-ulcerogenic agent, at least against gastric ulcers, appears justified and worthy of further study.

One of us (N.N.) would like to acknowledge financial support from Reckitt and Colman PLC whilst reading for a Ph.D degree at the University of Aston in Birmingham. We all acknowledge the help of Geest Industries (Peterborough) and Professor A.K. Sanyal (College of Medical Sciences, Baranas Hindu University, Varanasi, India) in supplying banana powder, and colleagues, especially Dr M.D. Day, of Reckitt and Colman PLC for arranging the supplies of banana powder and for helpful discussions.

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(Received August 10, 1983.
Revised November 30, 1983.)