DIETARY FIBER AND SHORT CHAIN FATTY ACIDS IN THE MANAGEMENT OF PERSISTENT DIARRHOEA.

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AND OTHER

1995 – 009

(FACE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B. Date

Principal Investigator DR.G.H.RABBANI/DR.G.FUCHS Trainee Investigator (if any) DR. ? TEKA

Application No. 95-009 Supporting Agency (if Non-ICDDR,B)

Title of Study Effects of dietary fibres and short-chain fatty acids in the management of children with persistent diarrhoea.

Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects Yes No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship Yes No
2. Does the study involve:
   (a) Physical risks to the subjects Yes No
   (b) Social Risks Yes No
   (c) Psychological risks to subjects Yes No
   (d) Discomfort to subjects Yes No
   (e) Invasion of privacy Yes No
   (f) Disclosure of information damaging to subject or others Yes No
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) Yes No
   (b) Use of fetal tissue or abortus Yes No
   (c) Use of organs or body fluids Yes No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study Yes No
   (b) Procedures to be followed including alternatives used Yes No
   (c) Physical risks Yes No
   (d) Sensitive questions Yes No
   (e) Benefits to be derived Yes No
   (f) Right to refuse to participate or to withdraw from study Yes No
   (g) Confidential handling of data Yes No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No
5. Will signed consent form be required:
   (a) From subjects Yes No
   (b) From parent or guardian (if subjects are minors) Yes No
6. Will precautions be taken to protect anonymity of subjects Yes No
7. Check documents being submitted herewith to Committee:
   ✓ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   NA Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   NA Questionnaire or interview schedule *

   * If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
   1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
   2. Examples of the type of specific questions to be asked in the sensitive areas.
   3. An indication as to when the questionnaire will be presented to the Cttee. for review.

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator Trainee

DIETARY FIBER AND SHORT CHAIN FATTY ACIDS IN THE MANAGEMENT OF PERSISTENT DIARRHOEA.

Principal Investigators:	G.H. Rabbani, MD,MSc,DPH,FACG Scientist.
	George Fuchs, MD. Acting Director, CSD.
Co-Investigator:	Telahun Teka, MD. Research Fellow
Co-investigator:	Asma Islam, MD,MPH Scientist
Starting Date:	As soon as approved (? February 1995).
Completion Date:	2-years from the date of approval.
Total Budget Required:	US $ 63,435

G Fuchs MD

Signature
Director, Clinical Sciences Division.

Date 10 April 1995

ABSTRACT SUMMARY :

Although persistent diarrhoea is recognized as an important cause of malabsorption, diarrhoea, growth failure, and mortality in children, a satisfactory form of treatment either by diets or pharmacological agents has been elusive. A wide variety of different compositions of diets have been tried but without the expected improvements. In this regard, a basis exists to consider dietary fiber as a potentially important adjuvant in a therapeutic regimen because of their beneficial effects on large and small bowel morphology and function. And while dietary fiber have been found to be effective in many types of gastrointestinal disorders, their role in persistent diarrhoea has not been investigated. We believe there exists a convincing body of evidence providing a rationale to study dietary fibers and SCFAs in children with persistent diarrhoea. We therefore propose a controlled clinical trial in children with persistent diarrhoea in which we compare diets supplemented with pectin (a fiber known to generate high quantities of SCFAs), green banana (on the basis of our preliminary observations), or an unsupplemented diet. If found effective, specific dietary fibers have the potential to be a simple, inexpensive, safe, culturally acceptable, and accessible therapeutic modality in the management of persistent diarrhoea.

1. HYPOTHESES

1. SCFAs are the major source of energy for the colonocytes and are known to produce beneficial effects on the structure and functions of the colon specifically affecting colonic mucosal transport of salt and water. SCFAs also have anti-inflammatory and antimicrobial properties against enteric pathogens. We hypothesize that these properties of SCFA will result in reduced stool volume and shortened duration of diarrhoea in children with persistent diarrhoea.

2. Short-chain fatty acids (SCFA) formed in the colon by the anaerobic fermentation of unabsorbed pectin will promote small bowel repair probably by enteric neurohumoral mechanisms in children with persistent diarrhoea.

3. Pectin (a dietary fiber that is a good source of SCFA) provided to children with persistent diarrhoea will result in reduced stool volume and shortened duration of diarrhoea.

4. Cooked unripe banana is a good source of pectin and will result in reduced stool volume and shortened duration of diarrhoea in children with persistent diarrhoea.

2. OBJECTIVES

1. Determine the association of fecal SCFA with small bowel permeability, nutrient absorption, stool volume, and duration of diarrhoea in children with persistent diarrhoea.

2. Compare the effect of pectin versus no supplementation on small bowel permeability, nutrient absorption, stool volume, and duration of diarrhoea in children with persistent diarrhoea.

3. Compare the effect of cooked unripe banana versus no supplementation on small bowel permeability, nutrient absorption, stool volume, and duration of diarrhoea in children with persistent diarrhoea.

3. BACKGROUND

Persistent diarrhoea (PD) is defined as having frequent (>5/day) loose stools for more than 14 days. Persistent diarrhoea is a problem of major importance in children in developing countries (1). While oral rehydration therapy programs are successful in controlling deaths from acute dehydrating diarrhoeas, deaths due to persistent diarrhoeas are found to be overt and of increasing significance (2). And although persistent diarrhoea accounts for 10% of diarrhoeal disease in children, it accounts for 50% of all childhood deaths due to diarrhoea (2). Further, malnourished children in Bangladesh have a 68-fold higher risk of death from persistent diarrhoea compared to better nourished children of the same community (3). The intimate association of persistent diarrhoea with malnutrition in children emphasizes the urgency for appropriate interventions to reduce immediate as well as long term complications. A relatively intact and normally functioning gastrointestinal tract is obviously imperative for optimal nutritional rehabilitation of these children.

The pathophysiological mechanisms of persistent diarrhoea are often unclear, but include a complex combination of host and at times microbial pathogenic mechanisms. Small bowel mucosal abnormalities which are nearly universal in children with persistent diarrhoea are also incompletely understood, but probably are the result of several abnormal conditions acting in concert. The original intestinal insult that initiates the process of persistent diarrhoea is believed by most investigators to often have been superseded by ensuing complications by the time the child comes to medical attention. These would include complications such as protein-energy malnutrition, micronutrient deficiencies, and a hostile small bowel environment which are all thought to play central roles in the mechanism of the disease(4).

Other than certain features that all children with persistent diarrhoea have in common, the spectrum of specific clinical abnormalities and pathogenic mechanisms has made optimal management of persistent diarrhoea difficult and often unclear. However, the major therapeutic intervention in persistent diarrhoea continues to focus on prompt and effective nutritional management. This approach emphasizes the correction of the underlying intestinal abnormalities and related malnutrition, regardless of the initial inciting cause, as the best method to achieve recovery. Both parenteral and enteral nutrition have been widely used in the developed world in the management of persistent diarrhoea (5). In the developing world, inexpensive, culturally acceptable, and effective forms of nutritional therapy which might also be suitable for use at the community level are crucially needed.

During the last decade, the beneficial role of dietary fibers in the management of a variety of gastrointestinal disorders has been the subject of increased interest. Many types of fibers including wheat bran, oat bran, cellulose, guar gum, ispaghula, and pectin have been shown to be useful in the symptomatic control through a variety of mechanisms in inflammatory bowel disease, diversion colitis, diabetes mellitus, gall stone disease, among others (6,7). It is relevant that dietary fiber may have a role in the pathogenesis of persistent diarrhoea and may have similar beneficial therapeutic effects.

Of particular interest, and we believe of potential relevance to childhood persistent diarrhoea, it has been postulated that the therapeutic effects of fibers in certain diseases are mediated by the effect of short chain fatty acids (SCFAs) produced in the colon on small bowel structure and function. SCFAs (mainly acetate, propionate, and butyrate) are generated in the colon by bacterial fermentation, especially by anaerobic organisms, of dietary fibers, polysaccharides, and sugars that escape absorption in the small bowel. SCFAs have been determined to be an important source of energy for colonocytes (chiefly *n*-butyrate), and that they play a significant role in the overall regulation of colonic function including fluid and electrolytes transport, protein synthesis, and antibacterial actions (8,9). Studies by Ramakrishna et al (10) showed that SCFA concentrations are significantly reduced in cholera and we have demonstrated that (unpublished) SCFAs have therapeutic properties in the treatment of shigella colitis in animals.

While the physiologic and therapeutic importance of SCFAs as related to the colon have been appreciated for some time, recent evidence in animal models indicate that the influence of SCFA is not limited to the large intestine but is also important to the small bowel as well. SCFAs have been shown to promote small intestinal hyperplasia (11) resulting in an increase in absorption of nutrients, fluids, and electrolytes. A recent innovative study in rats in which the colon was surgically disconnected from the small bowel has shown that SCFAs infused into the caecum stimulate small bowel mucosal growth as evidenced by increased jejunal DNA, villous height, surface area, and crypt depth. This jejunotrophic effect of colonically infused SCFAs was mediated by the autonomic nervous system and was associated with increase jejunal gastrin, and perhaps peptide YY (PYY)(12). In another study in rats with massive small bowel resection, those that did not receive metronidazole (and who therefore had normal colonic flora) had better weight gain, carcass protein, nitrogen balance, as well as small bowel mucosal dry weight, protein, and DNA compared to rats who received metronidazole (13). In this study, decreased colonic fermentation as measured by caecal SCFAs reduced small bowel intestinal adaptation and nutritional recovery. Although the number of studies are limited to date, they clearly indicate SCFAs generated in the colon to have direct trophic effects on the large bowel and small bowel mucosa.

In most developing countries, including Bangladesh, a significant proportion of diarrhoeal children have carbohydrate malabsorption (14). Children with persistent diarrhoea especially might be predicted to benefit from treatment with dietary fibers, however this needs to be evaluated and confirmed under protocol. Within this context, it is also of interest that our uncontrolled trial at ICDDR,B hospital indicated that a fibre rich diet (cooked unripe banana) reduced the diarrhoeal stool and improved symptoms in children with persistent diarrhoea (Table 1).

Rationale for pectin

Pectins are the polysaccharide nonstructural components of plant cells (cell wall, intracellular layers) which are found in relatively high concentration in the pulp of citrus fruits, apples, beets, and carrots. The physiological, morphological, biochemical and functional effects of pectin on small and large bowels of humans and animals has attracted much attention. Compared to other dietary fibers, pectin is soluble and almost completely metabolized. Fermentation by colonic bacteria yields SCFAs in their passage through the colon, a certain amount of which are excreted as part of the fecal solute. Among the different fibers, pectin in particular is known to generate high quantities of SCFAs. The effect of pectin on the utilization of minerals and trace elements in human beings is minimal as compared to its major effect on the metabolism of lipids and carbohydrates. The few studies in this regard reveal that absorption of calcium, magnesium, copper and zinc utilization are either unchanged or reduced in the presence of pectin (15,16)

Rationale and preliminary data for the use of unripe banana

Green, unripe plantains have been reported to have a protective capacity against acute aspirin-induced gastric mucosal injury in rats (20). Banana powder also has been demonstrated to protect against aspirin, indomethacin, phenylbutazone, prednisolone, and histamine induced ulcers in guinea pigs (21). Acute gastric mucosal lesions induced by ethanol and indomethacin are found to be protected by green sweet bananas in rats although the protection was incomplete and temporary in chronic ulcers in this study (22). Beneficial effects of the administration of dried banana powder to patients with non-ulcer dyspepsia and peptic ulcer has also been observed (23,24). It has been hypothesized that bananas enhance mucosal resistance and promote healing of ulcers due to the presence of water-soluble polysaccharides (eg pectins) in unripe plantains and surface-active phospholipids in ripe sweet bananas (24).

Approximately 75% of resistant starch (25), i.e. starch that escapes digestion in the small intestine, in banana origin undergoes fermentation in the colón in humans (26). Anaerobic breakdown of these starch yields short-chain fatty acids, CO_2, H_2 (methane production minimal) and energy. In addition, bananas contain pectin, phospholipids and other ingredients which have the potential to directly affect the physiological and biochemical functions of humans and animals.

Green banana has been used as an antidiarrhoeal agent in many traditional communities of Asia and Africa. We have conducted a preliminary study (unpublished) of 30 children with diarrhoea for more than 14 days admitted to our paediatric treatment unit. The diarrhoea was associated with nutrient malabsorption in most children although a few (3/30) had infectious gastrointestinal disease due to *Vibrio cholerae* non-01 and *Salmonella* group B. The nutritional status of the children varied from 40% to 65% of the median weight for age of the NCHS standard. The children were sequentially provided with one of two diets depending on the refractory nature of the diarrhoea; a milk-based diet (milk suji, 72 kcal/l) or a rice-based diet (rice suji, 44 kcal/100 ml). Unripe banana was boiled, peeled, and blended with each type of diet (200 g banana/l) and was fed to the children every 1-2 hours. The results indicated that the banana-diet significantly improved the stool consistency and reduced stool volume and duration of diarrhoea compared to banana-free diet (**Table 1**). It could be speculated that the mechanism of action of banana might be related to its high content of fibre (6.45%).

Table 1. Preliminary Observations of Effect of Unripe Banana on Persistent Diarrhoea*

	Control $n = 10$	Rice suji + Banana $n = 10$	Milk suji + Banana $n = 10$
Age (mo)	7.4 ± 3	7.6 ± 2	8.1 ± 2
Change from watery to soft stool (days)	5.8 ± 1.7**	2.3 ± 0.4	2.3 ± 0.6
Duration of hospital stay after starting banana (days)	7.9 ± 3.1**	3.1 ± 0.5	2.5 ± 0.5
Volume of stool post-banana (*l*)	3.1 ± 1.4**	0.5 ± 0.1	0.7 ± 0.6

*Data presented as mean ± SD.
**Control significantly different from other groups, $p < 0.05$.

In summery, we believe that there is convincing evidence providing a rationale to study dietary fiber and SCFAs as a potential inexpensive, safe, culturally acceptable, and accessible therapeutic modality in children with persistent diarrhoea. We therefore propose the current study to evaluate the role of dietary fibers in the management of persistent diarrhoea in children by specifically examining the clinical effects of a fibre-enriched diet. Specifically, we propose a controlled clinical trial in children with persistent diarrhoea in which we compare diets supplemented with pectin (a fiber known to generate high quantities of SCFAs), green banana (on the basis of our preliminary observations), or unsupplemented diet.

4. MATERIALS AND METHODS

4.1 Study design and sample size.

This study will be a controlled clinical trial of 45 children less than 5 years old with persistent diarrhoea who will be assigned by block randomization into one of three diet groups: dietary supplementations with either raw banana (n=15) or pectin (n=15), or the control diet of no supplementation (n=15). The sample size estimation is based on an assumption of at least 40% improvement in the treatment groups at a 5% significant level with 90 % statistical power. The children will be selected from the outpatient department of the ICDDR,B hospital and will be admitted to the study ward. The effects of treatment will be assessed by clinical and biochemical parameters related to the gastrointestinal tract.

4.2 Patient Inclusion and Exclusion Criteria

Inclusion Criteria

-- Age equal to or older than 5 months and less than 5 years. Only male children will be studied because of the difficulty of separating urine from the stool in female children.

-- Diarrhoea lasting for 14 or more days within the previous 3 weeks, with average number of stools greater than 5 per day.

-- The ability of parents or legal guardians of the child to provide voluntary, informed consent for their child's participation in the study.

Preference will be given to those children who are living within a short distance from the ICDDR,B hospital for the purpose of follow up after discharge from the hospital.

Exclusion criteria

-- Immediate life-threatening infection or sepsis.

-- Unstable medical condition that is likely to result in the patient being unable to complete the 20 days of the trial.

-- Inability to tolerate enteral feedings.

-- Bloody stools.

-- Concomitant diagnosis of other chronic illnesses including tuberculosis, malaria, hepatitis, etc.

-- Severe malnutrition (less than 40% of median weight for age by NCHS standard).

-- Refusal of voluntary consent by the parents.

4.3 Clinical Management: After admission into the study, a complete history of the present illness will be obtained and a physical examination done by a physician. The child will be placed on a "cholera bed" to facilitate quantitative collection of stool and urine. Urine will be collected separately from stool by applying polyvinyl urine collection (PUC) bags over the urethra. A physician will evaluate the patient twice each day to specifically assess the severity of a set of defined clinical symptoms. All stool will be collected in a plastic lined bed with the frequency recorded by the attending mother or the nurses. Patient status, body weight, weight of the stool and urine, and physical characteristics of the stool will be assessed and recorded by nurses experienced in research protocols.

Treatment with antibiotics including metronidazole, will be prescribed only when it is bacteriologically or clinically indicated.

4.4 Study Diets: All children will be fed meals of boiled rice, fish, meat, boiled potato, refined white bread, egg, and milk. The young children below 1-year's of age (5-12 months) will be given infant formula or a rice or chicken based diet. During the study known amounts of food will be given with their known calorie contents. Since the plant fibers of many vegetables normally consumed by the patients may confound the effects of the dietary intervention, the children will not be given vegetable containing food, particularly curreys containing leafy vegetables during the study period. Vitamins and mineral supplements to meet 100 % RDA (recommended daily allowance) will be provided daily. The diets will be standardized according to fibre content (apart from interventions of pectin or unripe banana) and the proportion of carbohydrate, fat, and protein. A glucose-polymer will be added to the diet of "no-fiber" group to compensate for the fiber-specific calories.

The amount of different fibers including pectin that have been used by various investigators in the treatment of inflammatory bowel disease ranged from 10g to 40g per day (24-26). On the basis of this dose, we calculate that a dose of pectin 4 g/kg body weight would be sufficient for the children aged between 5 months to 5 years.

For the children in the unripe banana group, unripe banana will be cooked, peeled, and the pulp blended using a standard kitchen blender. Hundred gram of banana pulp (wet weight) will be mixed with 1000 ml of a milk-based or rice-based diet and will be offered freely (ad libitum) to children. The blended banana pulp with small quantities of salt and sugar for improved palatability will be given to the children freely. The amount of pulp to be provided is the same amount we have previously used with good results in our preliminary study. The approximate macronutrient composition of banana is carbohydrate 29 g, protein 1 g, and fat 0.2 g with an energy value of 122 kcal per 100 g.

The control group of children will receive the regular diet without supplemental pectin or unripe banana.

4.5 Anthropometry: In addition to the daily weights, the following anthropometric measurements will be obtained on admission and weekly: weight, length, weight for length, body mass index (kg/m^2), skinfold thickness measured by skin calipers (triceps and subscapular), midarm circumference, and compared to the standards according the National Center for Health Statistic data as well as standards for Thai children (27,28). BMI (body mass index) will be compared to the standardized curves of the National Health and Nutrition Examination study while skinfold thickness and midarm circumference will be compared to the standards of Karlberg, et al.(29,30). The childrens' nutritional status (normal or mild, moderate, severe acute or chronic malnutrition) will be classified according to the scheme of Waterlow and assessed by Z score (31,32).

4.6 Laboratory Investigations: After clinical assessment of the patient, stool and rectal swab specimen will be taken for isolation of enteropathogens including Shigella, Salmonella, V. cholera, E. coli (enterotoxigenic, invasive, adherent, haemorrhgic, aggregative) and rotaviruses. Fresh specimens of stool will also be examined for the detection of ova and parasites. Patients will enter the study only after treatment of identified bacterial pathogen and parasite who continue to have diarrhoea. Venous blood sample (3 cc) will be taken for estimation of serum electrolytes, glucose, and differential count.

4.6.1 Metabolic balance studies: The effects of the dietary interventions on macronutrient and energy absorption will be evaluated by 72-hour balance studies in a subsample of 10 children from each of three study groups. Fecal fat will be quantitated by the van de Kamer method (33), nitrogen in urine and faeces will be determined by the micro-Kjeldahl method (34), and faecal energy by adiabatic bomb calorimetry (Gallenkamp) (35); faecal carbohydrate is estimated by the difference. Apparent energy, fat, and carbohydrate absorptions are then estimated from the intake minus faecal excretion. To calculate apparent nitrogen retention, faecal and urine losses were subtracted from the nitrogen intake (36). All absorption and retention data of the macronutrients will be computed with the actual composition of each diet. In a subgroup of children, the 72 h balance study will be done on days 4-6 to compare the results with earlier studies (days 3-5).

4.6.2 Faecal short chain fatty acids: Stool samples (50 g) will be obtained by catheter on day 1,2,3, and 7 and at discharge for the determination of SCFAs in stool. Specimens will be stored at -40° C until analysis. Magnesium chloride ($MgCl_2$) will be added to the stool sample as a bactericidal agent to prevent fermentation during storage (37). The following SCFAs will be determined by gas chromatography acetate, propionate, butyrate, valerate, and isovalerate. Briefly, homogenates of two to ten g faecal sample is prepared by the vacuum-distillation method described by Zijlstra et al and modified by Treem et al (38,39). The distillate is subsequently analyzed by gas-liquid chromatography using a gas chromatograph [Hewlett-Packard (HP) Model #5890, Palo Alto, CA, U.S.A.] attached to an integrator (HP 3396 series II). Different concentrations of SCFAs are prepared by using acetate, propionate, butyrate, valerate, and caproate which are utilized as internal standards. All determinations of faecal SCFAs will be run in triplicate and the mean expressed as the final value. The concentration of non-fermented fibers in the stool will be determined using total dietary fiber assay kit (Sigma Chemical Company, St.Louis, MO).

4.6.3 Faecal alpha$_1$-Antitrypsin (alpha$_1$-AT): Excessive plasma protein losses via the gastrointestinal tract has a variety of causes, but perhaps occurs most commonly with inflammatory intestinal diseases, some of which might be relevant in persistent diarrhoea. The quantity of the plasma protein alpha$_1$-AT in stool has been used as an endogenous probe of enteric protein loss (40). Faecal alpha$_1$-AT will be expressed in terms of concentration (ie, per gram of stool) and for this reason a random stool specimen can be used for determination. The faecal concentration of alpha$_1$-AT will be determined by radial immunodiffusion using aqueous extracts of lyophilized stool. A fecal alpha-$_1$ AT clearance test will be done by taking a serum sample during the 72 h balance study period (41, 42).

4.6.4 Intestinal permeability test: The effects of the dietary fibers on the small bowel mucosal integrity will be assessed by performing lactulose-mannitol intestinal permeability test (IPT). Differential sugar tests have been recommended as the most appropriate non-invasive means of investigating small intestine permeability (43,44). This test is based on the simultaneous administration of two probe molecules, a monosaccharide and a disaccharide, and subsequent estimation of the urinary recovery of each molecule. In disease of the proximal small intestine the recovery of the monosaccharide is reduced, perhaps due to a reduction in absorption surface area, while that of the intact disaccharide is increased. The result is expressed as a ratio. A significant advantage of the differential sugar permeability test is that the effect of extraneous variables that influences a single test sugar such as d-Xylose can be overcome by expressing absorption as a ratio of absorption of the two markers (45). Variables such as adequacy of ingestion, gastric emptying rate, small bowel bacterial overgrowth, intestinal transit time, and renal clearance do not alter the absorption since they affect both markers equally (46). Abnormal permeability is also associated with diarrhoeal disease and with mucosal damage, thereby providing a reliable and useful index of mucosal integrity (47).

Briefly the procedure is as follows. A baseline urine is obtained and the child fasted for at least 2 hours prior to testing. The child is then given the test solution containing 1 gram of mannitol and 5 grams of lactulose in 20 milliliters of 1% chloroform water. Urine will be collected for the following five hours and preserved with one drop of 20% wt/vol chlorhexidine gluconate. Aliquots will be stored at -4^{0}C until testing. Urine lactulose and mannitol will be quantitated by enzymatic assay methods.

4.7 Additional outcome variables:

In addition to the above, treatment effects will be evaluated by the following variables:

-Duration of diarrhoea (the number of hours from the start of the intervention until excretion of the last liquid or semi-liquid stool not followed by another abnormal stool within 24 hours).

-Time to resolution of diarrhoea (that time when there is 2 or less formed stool during any 24-hour preceding period).

-Stool volume and weight

-Consistency of the stool. Liquid stool is defined as when faecal material takes the shape of the container and the material can be easily transferred without sticking. The term "soft stool" is defined as being more thick, takes the shape of the container but cannot be poured easily. "Formed stool" retains its shape, does not stick to the container, and is characterized by the absence of mucous.

-Therapeutic failure will be defined as recurring dehydration >5% using clinical criteria[1] or faecal excretion >20 g/kg body weight by day 7.

4.8 Management of dropout cases:

If the patient leaves the study after randomization and before completion, the data will be excluded from the analysis and the case will be replaced by a new entry from the randomization list. If a patient develops clinical complications (severe hyponatremia, septicemia, intestinal obstruction, paralytic ileus, etc) which prevent continuation of fibre supplementation, the patient will be withdrawn from the study and replaced with a new entry.

4.9 Organization of the trial.

Patients will be selected from the outpatient department of the ICDDR,B and will be admitted to the study ward if they fulfil the admission criteria. The principal investigators with the assistance of a research fellow will take care of the patients for clinical management. The research data will be recorded in coded forms and will be preserved separately after completion of the study. Patients will be admitted into the study during the morning hours

(8:30 am to 12 noon) to enable a convenient 8- hourly schedule and to facilitate recording of the clinical events.

5. SCHEDULE OF TESTING

After admission of the patients to the study ward, the following tests will be determined according to the schedule below. This will avoid overlap and leave time for a direct effect of the intervention we are looking for.

	Hospital Day										
ACTIVITY	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Dietary Management											
Regular diet	X	X	X	X	X	X	X	X	X	X	X
Fiber intervention (pectin/banana)		X	X	X	X	X	X	X	X	X	X
Clinical Assessment											
Stool volume, weight, freq	X	X	X	X	X	X	X	X	X	X	X
Anthropometry*	X							X			X
Intake	X	X	X	X	X	X	X	X	X	X	X
Laboratory											
Fecal SCFA		X	X	X	X	X	X	X			
Fecal α-1 AT		X						X			
IPT		X						X			
Metabolic balance study				X	X	X					

*Body weight assessed daily.
SCFA, short chain fatty acids; α-1 AT, alpha-1 antitrypsin; IPT, intestinal permeability test.

6. DATA ANALYSIS

Efficacy Analysis

Efficacy analysis will be based on:

1. Change in volume, weight, and/or frequency of diarrhoea assessed by continuous stool collection.

2. Change in body weight.

3. Change in daily caloric intake, fluid intake, and urine output.

4. Changes in macronutrient and energy absorption together with mucosal integrity and permeability

The results of these analyses will emphasize descriptions of parameter values for the three groups. Secondarily, it will compare the values using analysis of variance procedures. Analogous nonparametric procedures may be used if the usual assumptions of analysis of variance are violated. The change from baseline in efficacy parameters during the intervention and post-intervention (7 days after discharge) phases will be investigated.

7. ETHICAL CONSIDERATION

This study will be done in children more than 5 months but less than 5 years of age who will be provided with a diet that is supplemented with boiled green banana or refined pectin. Both these substances are constituents of normal food and the potential for adverse effects is very low but would include abdominal distension. The metabolic balance study will require the child to be restrained, however no additional invasive clinical procedures are planned as part of the study protocol. The clinical record of each patient will be kept confidential and the final report will be published without reference to the identity of the individual.

ABSTRACT SUMMARY

The primary objective of this protocol is to evaluate the therapeutic efficacy of pectin and banana in the dietary management of children with persistent diarhoea. Forty-five male children of age between 5 months and 5 years with persistent diarrhoea (frequent loose stools for 14 days or more) will be selected from the ICDDR,B hospital and will be randomly assigned to three dietary groups, in which the normal diet will be supplemented with either pectin, banana, or no supplementation. The following procedures will be observed.

1. Before admitting the children into the study, voluntary informed consents will be taken from the parents or the gurdians of the children. The parents of the children will have the right of accepting or refusing admission into the study and will be allowed to withdraw from the study at any point after admission.

2. The children will stay for 7-10 days in the hospital. During this time, stool, urine, and vomit will be collected and measured for the purpose of the study. For the metabolic balance study, 72 h stool and urine will be collected after giving the child a dose of charcoal as a non-absorbable marker. Medicated charcoal tablet, has been used as a standard form of safe and useful diagnostic marker in the studies of GI functions. The children will receive normal milk-based or rice-based diets, supplemented either with pectin or banana. Small amount of blood (1 cc) will be taken 2 to 3 times during the study for clinical purposes. These will not be hazardous in any way for the patient.

3. No other investigational drugs will be given to children except the standard therapy for the type of diarrhoeal illness the child is suffering from.

4. Confidentiality of the personal and clinical information will be maintained throughout study period. Data files will be coded and stored in diskette which will only be accessible to the responsible investigators.

5. After completion of the study, the results will be published without reference to the name and identity of the individual patients.

6. If these dietary intervantions are found to reduce the duration of diarrhoeal illness or decrease the volume of purging or improve the quality of the diarrhoeal stool, this would provide an important but simple means of dietary management of persistent diarrhoea in children.

7. Both banana and pectin are considered as useful food items and is consumed freely without any unpleasant effects. Pectin is also used in the food industry as binding agent, particularly in confectioneeries. We do not expect any side effects form these items when given in children within acceptable dose ranges.

8. In this study, the following materials will be used:

Patient's clinical record of medical data, record of laboratory investigations — stool, urine and blood samples. No use of organs, tissues, fetus or abortus will be made.

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8. DETAILS OF BUDGET

	Budget for 2 years	
Personnel		
G. H. Rabbani (PI, 30% salary for 2 years		$ 4,000
George, Fuchs, M.D (P.I No cost to ICDDR,B)		000
Telahun, Teka, M.D. (Co-investigator, Res. Fellow, 70%)		4,000
Asma, Islam, M.D. (Co-investigator, No cost)		000
Clinical Res, Assistant, M.D (100% to be hired)		3,000
Hospital Attendant, GS-1 (to be hired)		1,000
Lab. Technician (for IPT, SCFA, a_1 -AT), (50% per year)		4,200
	Sub-Total	S 16,200
Patients Hospitalization		
50 pts * 10 days @ $ 16/day		8,000
Follow up visits for pts		1,000
	Sub-total	S 9,000
Laboratory Tests		
Stool cultures for pathogens		2,000
Stool ME, Serum electrolyte, blood CBC		2,000
SCFA tests in stool		2,000
Lactulose/Mannitol tests		1,000
Fecal Fat 51.70 * 30 tests		1,551
Fecal N_2 11.50 * 30 tests		345
Fecal calorie 9.65 * 30 tests		289.50
Diet Analysis for Fat, N_2 and calorie		365
72-hour urinary N_2 analysis		345
TBARS (blood) 30 samples		1,000
	Sub-Total	10,895.50
Drugs and Reagents		
Pectin from Sigma, USA		500
Raw banana		200
Fiber Test Kits Sigma		1,000
Charcoal tablets/Acetic acid		200
	Sub-Total	1,900

Equipment and supplies	
Child weighing Scale	1,000
Food processor (Heavy Duty)	1,000
Stool collection bags (polyethylene)	300
Urine collection bags (puc)	300
Sub-Total	2,600
Travel	3,000
Logistics	
Xerox/paper etc	500
Fax/DHL/Reprint	2,000
Dry ice	200
Stool Shipment for SCFA	2,000
Manuscript preparation	500
Sub-total	8,200
Direct cost $	$ 48,795.50
30% Indirect cost $	S 14,638.65

Total Cost	US$ 63,435.15

International Centre for Diarrhoeal Disease Research, Bangladesh
GPO BOX 128, Mohakhali, Dhaka 1212

PARENT'S CONSENT FORM

Evaluation of dietary fibre and raw banana in the clinical management of persistent diarrhoea in children.

1. I Understand that my child has developed persistent diarrhoea, i.e, frequent loose stools for more than 14 days. I also understand that peristent diarrhoea is difficult to treat and the child may develop nutritional complications including malabsorption and growth failure. I was also informed that the International Centre for Diarrhoeal Disease Research, Bnagladesh is carrying out research activities to indentify suitable means of dietary management of persistent diarrhoea in children less than 5 years of age.

2. In the present study we are evaluating the therapeutic effects of green banana and pectin in the management of persistent diarrhoea in children.

3. Your child will be admitted into the hospital study ward for 7-10 days. During this period the child will be given the standard treatment for the diarrhoeal illness, which may include normal feed, ORS, IV fluid, and drugs.

4. The child will be fed the standard hospital diet during the illness. Depending on the age, the child will be given either a milk-based, rice-based, or chicken-based diet. In addition he will be given pectin at a dose of 4g/kg body weight per day or green cooked banana (*Kacha Kola*), 100-200 g per day. Your child will be randomly assigned to any of the three different diet groups and there is equal chance that the child will get either pactin or banana or only the regular diet without pectin and banana.

4. As you may know that different preparations of cooked green banana (curry, bharta, chop etc) is normally eaten in this country. Your child will be given a similar preparation of green cooked banana which will be mixed with the regular diet and fed freely during the period of illnesss. The amount of cooked banana that will be given is 100-200 g per liter of the feed, i.e, approximately half to one full banana per child per day. We do not expect any unpleasant effects due to feeding banana in children above the age of 5 months.

5. Pectin is present in large quantities in most unripe fruits including mango, guava, orange, apples, and banana. Pectin is a complex carbohydrate and is freely used in the industrial preparations of confectioneries. Your child will be given pectin at a dose of 4g/kg body weight per day in 4-6 divided doses. The pectin powder will be dissolved in water and will be given by mouth. The solution is tasteless or slightly salty.

6. During the study, all stools, urine, and vomit will be collected and quantitated daily. Daily body weights and heights will be measured. One ml (1 ml) of blood

will be collected 2-3 times during the entire illness period for measuring electrolytes, and other serum chemistries.

7. If you have your child admitted into the study, you will still possess the right to withdraw from the study at any point in time. In that case, You will not be penalized anyway and your child will not be prevented from getting standard care of the hospital.

8. All medical records of your child's treatment will be kept confidential and will be published without reference to the name and identity of the child.

9. If you are confident that you have understood all these points and all your questions have been answered to your satisfaction, and you wish to have your child admitted into the study, plese indicate your consent by signing your name or thumb printing in the space below.

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Signature of the Investigator. **Signature of Parent/Gurdian**

Date:________________________ Date:______________________

Patient's Name______________________**Adm #**__________**Date**________

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ
জি পি ও বক্স ১২৮, ঢাকা, বাংলাদেশ

অভিভাবকের সম্মতি পত্র

উদরাময় ও রোগ গবেষণা-এর উদ্যোগে শিশুদের দীর্ঘমেয়াদী ডায়রিয়ার গবেষণা

আমি জানতে পেরেছি যে আমার বাচ্চার দীর্ঘমেয়াদী ডায়রিয়া হয়েছে, যেটা হলো ১৪ দিনের বেশী দিন ধরে ঘন ঘন পাতলা পায়খানা হওয়া। আমি আরও জানতে পেরেছি যে, দীর্ঘমেয়াদী ডায়রিয়ার চিকিৎসা করা বেশ কঠিন এবং এ থেকে অপুষ্টি দুর্বলতা শিশুদের বেশ ক্ষতি এবং মৃত্যুর ঝুঁকি বৃদ্ধি হতে পারে। আমি আরও জানতে পেরেছি যে বর্তমানে এই হাসপাতালে দীর্ঘমেয়াদী ডায়রিয়ার সুচিকিৎসার জন্য গবেষণা চালানো হচ্ছে, বিশেষ করে তিন বছরের কম বয়সের শিশুদের জন্য। খাদ্য পরিবর্তনের দ্বারা এ রোগের চিকিৎসার উদ্ভাবন করাই হচ্ছে এই গবেষণার মূল লক্ষ্য।

বর্তমান গবেষণায় সবুজ কাঁচা কলা ও পেক্টিন নামক এক প্রকার আঁশ জাতীয় খাদ্য উপাদানের উপর গবেষণা চালাচ্ছে। এই গবেষণা তিন বছরের কম বয়সের শিশুদের উপর যাহারা দীর্ঘমেয়াদী ডায়রিয়ায় আক্রান্ত হয়েছে তাদের উপর চালানো হবে।

আপনার শিশুকে এই হাসপাতালের গবেষণা ওয়ার্ডে ৭ থেকে ১০ দিনের জন্য ভর্তি করা হবে। এই সময় বাচ্চাকে ডায়রিয়ার রোগের উপযুক্ত চিকিৎসা দেয়া হবে যেমন, খাবার স্যালাইন, শিরায় স্যালাইন, ঔষধপত্র এবং পুষ্টিকর খাবার।

বাচ্চাকে হাসপাতালের ~~...~~ পুষ্টিকর খাবার দেয়া হবে। বয়স অনুযায়ী রোগীকে দুগ্ধ জাতীয়, ভাত-জাতীয় অথবা মুরগীর মাংস সম্বলিত খাবার দেয়া হবে। এছাড়া রোগীকে প্রতি কেজি ওজনের জন্য ৪ গ্রাম পেক্টিন এবং কাঁচা কলা সেদ্ধ (১০০-২০০ গ্রাম) প্রতিদিন খাওয়ানো হবে। আপনার রোগীকে তিন রকম

খাবারের মধ্যে যে কোন একরকম খাবার খুবই দেওয়া হত
খাবে হতো. যে কোন এক ধরনের খাবার কারণ সম্ভবত সমান
খাবে। আমাদের জেলীতে প্রোটিন, আমার টাটকা ফল অথবা
এমুনি ছড়া স্বাভাবিক খাবার দেওয়া হতো।

। আমি নিশ্চই বলেন যে আমাদের দেশে টাটকা ফলের
বিভিন্ন প্রকারের খাবার যেমন ডাল, ভাত, মাছ ইত্যাদি
প্রচলিত আছে। আমাদের বাচ্চাদের এই প্রকারের টাটকা ফলের
তৈরী খাবার দেওয়া হতো যেটা অন্যান্য সাধারণ খাবারের সাথে
পরিবেশন করা হতো। প্রতি বাচ্চাকে ১০০-২০০ গ্রাম টাটকা ফল
অথবা প্রতি ১ দিনের পরিমাণ খাবারের সাথে মিশিয়ে দেওয়া হতো।
এতে করে প্রতি বাচ্চাকে একটি অথবা অর্ধ পরিমাণ টাটকা ফল
দেওয়া হতো। প্রতি দিন। এতে টাটকা ফল থেকে আমাদের বাচ্চার
কোন প্রকার অসুবিধা হতো, বলে আমার মনে করি না।

। যথেষ্ট পরিমাণ প্রোটিন জাতীয় খাদ্য বিভিন্ন প্রকার টাটকা
ফলমূলের সাথে যেমন, টাটকা আম, পেয়ারা, কমলা, আনারস,
ইত্যাদি টাটকা ফল। প্রোটিন একটি প্রধান খাদ্য জাতীয় খাদ্য
যেটা কার্বোহাইড্রেট তৈরীর জন্য যথেষ্ট পরিমাণে প্রয়োজন
হয়। আমাদের বাচ্চাকে ৪ গ্রাম প্রোটিন প্রতি কে.জি ওজনের জন্য
দিনে ৪-৫ বার দেওয়া হতো। প্রোটিন পাউডার পানিতে মিশিয়ে
বাচ্চাকে খাওয়ানো হতো। এটির কোন খারাপ ফল হয় সম্ভবত
লোকের দেখা যেতো পারে।

। ভিটামিন এবং মিনারেলস জাতীয় সম্পূর্ণ খাওয়ার জন্য মাল, মুগ্ধ, ভাজী,
ইত্যাদি সংগ্রহ করা হত এবং এমুনি পরিমাণ করা হত শরীরের
জন্য। শরীর সারের দিক থেকে ১ এম.এল পরিমাণ করে
২ থেকে ৩ বার গ্রহণ করা হত ইলেকট্রোলাইটস এবং হাড়ের
গঠনের শরীরের জন্য।

যদি আপনি আপনার সন্তানকে এই গবেষণা প্রকল্পে ভর্তি করিয়ে আনেন তবু পরেও যেকোন সময় গবেষণা থেকে তাকে নাম প্রত্যাহার করিয়ে নিতে পারবেন। সেক্ষেত্রে আপনাদের কোনপ্রকার ক্ষতি পূরণ দিতে হবে না এবং আপনার সন্তানের স্বাস্থ্যগত চিকিৎসা এই হাসপাতালে কোন প্রকার ব্যাহত হবে না।

২। আপনার সন্তানের চিকিৎসার বিষয়ে সকল প্রকার তথ্যাদি গোপন রাখা হবে এবং তার নাম, পরিচিতি কখনও প্রকাশ করা হবে না।

৩। আপনি যদি মনে করেন যে এই গবেষণার বিবরণ সব বুঝতে পেরেছেন এবং আপনার সব প্রশ্নের সন্তোষজনক উত্তর দেওয়া হয়েছে এবং আপনি এই সিদ্ধান্তে পৌঁছেছেন যে আপনার সন্তানকে গবেষণায় ভর্তি করবেন তবে নীচে ও নাম সই করুন এবং বৃদ্ধাঙ্গুলির ছাপ দিয়ে আপনার সম্মতি প্রদান করুন।

ধন্যবাদ।

সাক্ষীর স্বাক্ষর—
তারিখ ______

পিতামাতা/অভিভাবকের স্বাক্ষর—
তারিখ ______

রোগীর নাম ______________ নং ____________ তারিখ ____________