Rapid cessation of acute diarrhea using a novel solution of bioactive polyphenols: a randomized trial in Nicaraguan children

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Goal We assessed the effectiveness of bioactive polyphenols contained in solution (LX) to restore normal bowel function in pediatric patients with acute diarrhea. **Background** While providing oral rehydration solution (ORS) is standard treatment for diarrhea in developing countries, plant-derived products have been shown to positively affect intestinal function. If a supplement to ORS resolves diarrhea more rapidly than ORS alone, it is an improvement to current care. **Study** In a randomized, double-blind, placebo-controlled cross-over study, 61 pediatric patients with uncontrolled diarrhea were randomized to receive either ORS+LX on day 1 and then ORS+water on day 2 (study arm) or ORS+water on day 1 and then ORS+LX on day 2 (control arm). Time to resolution and number of bowel movements were recorded. **Results** On day 1, the mean time to diarrhea resolution was 3.1 hours (study arm) versus 9.2 hours (control arm) ($p=0.002$). In the study arm, 60% of patients had normal stool at their first bowel movement after consumption of the phenolic redoxigen solution (LX). On day 2, patients in the study arm continued to have normal stool while patients in the control arm achieved normal stool within 24 hours after consuming the test solution. Patients in the control arm experienced a reduction in the mean number of bowel movements from day 1 to day 2 after consuming the test solution ($p=0.0001$). No adverse events were observed. **Conclusions** Significant decreases in bowel movement frequency and rapid normalization of stool consistency were observed with consumption of this novel solution.

Rapid Cessation of Acute Diarrhea Using a Novel Solution of Bioactive Polyphenols: a randomized trial in Nicaraguan children

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INTRODUCTION

Diarrhea is the second leading cause of death in children under the ages of 5 years in developing countries (Johansson *et al.*, 2009), a most concerning statistic as diarrhea may be prevented and treated. Acute diarrhea can lead to severe dehydration and electrolyte imbalance by loss of fluids, electrolytes, and nutrients (Munos *et al*., 2010). Oral rehydration therapy was initially developed to replace cholera-induced fluid loss (Pierce *et al*., 1969; Sentongo 2004), but has expanded to include diarrhea incited by other pathogens (Hirschhorn 1980; Nalin *et al*., 1979; Pizarro *et al*., 1983). The World Health Organization (WHO) standardized an oral rehydration solution (ORS) containing sodium, potassium, chloride, citrate, and glucose (Atia & Buchman 2009). Although ORS assists in diarrheal management, it does not reduce the duration of diarrhea or fecal volume (Canai *et al*. 2007). Instead, implementing ORS can increase stool volume in children during acute episodes (Sarker *et al*., 2001; El-Mougi *et al*., 1994). In order to optimize efficacy, the WHO recommended a modified ORS with reduced osmolarity, administration of zinc gluconate, non-digestible carbohydrates, rice powder, and probiotic

bacteria – all with mixed results (Gregogio *et al*., 2007; Basu *et al*., 2007; Narayanappa 2008; Hoekstra *et al*., 2004; Passariello *et al*., 2011).

In developing countries, attempts for rehydration using readily available household beverages often exacerbate intestinal fluid loss by elevating osmotic load and disrupting water and electrolyte absorption (Munos *et al*., 2010; Sentongo 2004). However, the proper use of ORS and public health measures in Nicaragua including widespread rotavirus vaccinations in infants has been associated with a 35% reduction in childhood mortality over 5 years in the early 1980s. This rate has since remained relatively constant (Gibbons, Dobie & Krieger 1994). Currently, antibiotics serve a very limited role in treating diarrhea in children and the utility of anti-motility agents is either contra-indicated or controversial due to heightened infection risks and adverse effects.

The use of naturopathic medicines in rural or developing populations is often attributed to the inaccessibility of western medicines for common infectious illnesses and a traditional belief in the natural, beneficial properties of plant and plant-derived products. Recent investigations into the efficacy of various plants have identified that their phytochemicals can affect intestinal function and motility (Njume & Goduka 2012; Bukhari *et al.*, 2013; Velazquez *et al*., 2012; Rajan *et al*., 2012; Patil, *et al*., 2012; Ezeja *et al*., 2012) and provide antibacterial activity (ABbassi & Hani 2012; Knipping, Garssen & van't Land 2012; Ismail, Sestili & Akhtar 2012; Mariita *et al.*, 2011; Assam *et al*., 2010). While commercial extraction and processing of these compounds can reduce their viability, a novel processed plant extract composition, LifeDrops (LiveLeaf Inc., San Carlos, CA), captures the bioactive potential of live plant cells. The LifeDrops solution contains a complete complex of green tea (*Camellia sinensis*) and pomegranate *(Punica granatum)* incorporating biologic co-factors key to delivering the full capability of the plants' immune response, termed LiveXtract solution (LX). The

mechanism behind LiveXtract solutions is based upon a transient polyphenol reaction common to nearly all higher plants. The site activation of this reaction by the body's enzymes delivers a powerful synergy of localized injury protection, toxin neutralization, and attenuation of inflammation that cannot be produced by conventional. polyphenol extracts (Romier *et al*., 2009; Vauzour, *et al*., 2010; Taylor, Hamilton-Miller & Stapleton 2005; Biasi *et al.*, 2011; Romier-Crouzet *et al*., 2009; Kim, Rajalah & Wu 2008).

The objective of this study is to compare the efficacy of ORS+LX (LifeDrops) versus ORS+water (placebo) in reducing the incidence and frequency of loose stools and associated gastrointestinal symptoms of pediatric patients with acute diarrhea in Nicaragua. We hypothesized that the addition of LifeDrops to standard ORS, compared to ORS alone, would reduce the time to normalization of stools and digestive function.

MATERIALS & METHODS

Study Design

This randomized, double-blinded, placebo-controlled cross-over study was conducted at a government-funded community health clinic in Managua, Nicaragua, between August and December 2010. Following torrential rains and flooding in the region from tropical storms Agatha and Matthew, there was a substantial increase in the incidence of consultations for acute diarrhea. With approval of the institutional review board of the Universidad Centroamericana de Ciencias Empresariales (IRB 2010013, registered ISRCTN57765025)), treatment-naïve, previously healthy pediatric patients between 2 and 17 years of age who arrived at the clinic with uncontrolled acute diarrhea within 48

hours prior to presentation were enrolled in the study. Written informed consent was obtained from the parents or legal guardians of patients who met the inclusion criteria.

Statistical Analysis

Sample size calculations were based on studies in acute diarrhea using standard ORS treatment in non-cholera pediatric patients. A sample size of $\geq$ 30 patients per arm was based upon detecting at least a 15% difference in the duration of diarrhea at the 5% significance level with 80% power. Differences between means of parametric data were analyzed with the Student's *t*-Test, with significance set at 0.05 level. Nonparametric data were analyzed with Chi-squared and Wilcoxon rank-sum tests.

Study Inclusion

All patients who presented to the clinic were assessed and included if they had acute gastroenteritis, including diarrhea, for 48 hours or less. Diarrhea was defined as three or more loose or liquid stool per day. Patients were excluded from the study if they had a history of uncontrolled emesis, grossly bloody stool, fever, clinical signs of a coexisting acute systemic illness (*e.g*., meningitis, sepsis, pneumonia), underlying chronic disease (*e.g*., heart disease, cystic fibrosis, diabetes), food allergies or other chronic gastrointestinal diseases, admitted use of probiotic agents in the previous 3 weeks or antibiotics or anti-diarrheal medication including over-the-counter and herbal substances in the previous 2 weeks, generalized cachexia, any signs of internal bleeding or drug abuse, or any condition assessed by standard of care to place unnecessary risk if placed on ORS alone. Every patient had a microscopic stool evaluation at the time of enrollment, and those positive for an intestinal protozoan infection were excluded from the study.

ORS+LX vs. ORS+water

After study eligibility was determined and consent was obtained, patients were randomized to one of two arms based upon a computer-generated random number listing. The study arm consisted of ORS+LX (LifeDrops) on day 1, then ORS+water on day 2. The control arm consisted of ORS+water on day 1, then ORS+LX on day 2. Patients in both arms were given one of the blinded solutions on the first day of clinical evaluation and subsequently monitored by clinic staff for two hours (Figure 1). A graduated dosing scale, based on patients' weight, determined the volume of LiveXtract solution administered (Table 1). In the control arm, the same volume of water was added to the ORS in order to equal the 25 mL total fluid volume given to patients in the study arm. To enhance the uptake of the test solutions, the ORS contained an added commercial artificial flavor and coloring produced by the Acama company in Central America. Zinc gluconate was not administered during the study period.

Cross-Over

Two hours after administration of either solution on day 1, the patients were released from the clinic with a maintenance amount of ORS for the next 24 hours. All patients were asked to return within 24 hours on day 2 for cross-over administration of the alternate solution.

Outcome Measures

The primary outcome measure was the time elapsed from the initial ingestion of ORS+LX or ORS+water to any subsequent "unformed" stool, based on the Bristol Stool Scale (BSS), a validated method of visually categorizing stool in 7 appearances based on stool shape and consistency. It has been shown to have reproducibility in pediatric cohorts (Lane *et al.*, 2011; Lewis & Heaton 1997). We considered any BSS > 4 to be "unformed" and $\leq$ 4 to be "formed." The clinical staff ranked the stool during the first 2 hours after solution ingestion and parents were trained to score and report the ranking of each bowel movement while away from clinic.

The secondary outcome measure was stool consistency based on BSS. Additional secondary outcome measures were defecation urgency and bloating/gas following fluid consumption, and a qualitative rating of abdominal pain (for patients able to comprehend and follow directions) on a numeric scale of 0 (none) to 10 (worst imaginable/continual) at 30, 60, 90, and 120 minutes after consumption of either solution on both day 1 and day 2.

RESULTS

Patient Demographics

A total of 61 patients were enrolled in this study with 30 patients randomized to the study arm (ORS+LX) and 31 patients to the control arm (ORS+water) on day 1. All subjects were found to be free of protozoan infection by microscopic stool examination, but the specific etiologies of their diarrhea were not definitely known, as per standard of care in this clinical care setting. The patients in each arm were comparable in age (mean age of 8 vs. 7 years, p=0.51) and weight (mean weight of 27 vs. 32 kg, p=0.31), but with more females present in the study arm and more males in the control arm (Table 2).

Response to Solutions Consumed on Day 1

The summary of results shown in Figure 2 demonstrates that patients in the study arm achieved a time-to-last unformed stool (a BSS ranking of 4 or less) in a mean elapsed time of 3.1 hours versus 9.3 hours among patients in the control arm (p=0.002) on day 1 of the study. In the study arm, 60% of the patients had their first bowel movement with a BSS of 4 or less after consuming the ORS+LX. In the control arm, only 29% of the patients had their first bowel movement with a BSS of 4 or less after

ORS+water consumption. At the second movement on day 1, 82% of patients in the study arm versus 35% of patients in the control arm reported stools with a BSS rating of 4 or less. ($p<0.001$)

Patients in the study arm also experienced a longer mean time between bowel movements after solution consumption: 3.7 hours in the study arm and 2.8 hours in the control arm, which did not achieve statistical significance. The mean time between the first and second bowel movements after consumption was 7 hours in the study arm versus 4.4 hours in the control group ($p=0.02$).

Response to Solutions Consumed after Patient Cross-over on Day 2

When patients returned on day 2 of the study, those in the study arm received ORS+water while those in the control arm received ORS+LX. After 2 hours, all patients in the study arm reported stool with a BSS rating of 4 or lower. Patients in the control arm subsequently reported resolution of their diarrhea at a rate comparable to that noted on day 1 for patients in the study arm (Figure 2). On day 2, patients in control arm had a mean ranking of stool of 4.5 prior to consuming the ORS+LX, which decreased to 3.2 by the first bowel movement after consumption and further decreased 2.2 by the end of day 2 ($p<0.01$). Patients given ORS+water on day 1 had a mean number of 4 bowel movements that declined to a mean of 2 after receiving ORS+LX on day 2 ($p<0.01$).

Secondary Outcome Measures

Patient-reported responses (e.g. abdominal pain) were incompletely collected during November and December of 2010, resulting in responses from only 10 study arm patients and 7 control arm patients, sample sizes too small for meaningful analyses. The rating of gas and bloating was comparable between the two arms over the two days, but patients in the control arm did report improvement in their levels of abdominal pain and urgency of defecation soon after consumption of the ORS+LX on day 2

(Figures 3 and 4). The rating of abdominal pain in patients in the control arm decreased to levels comparable to that reported by patients in the study arm within 2 hours after consumption of ORS+LX and was essentially identical to patients in the study arm at the end of the study period (Figure 3). The rating of defecation urgency, despite remaining unchanged for 24 hours after consumption of ORS+water, declined substantially within 60 minutes post-ORS+LX consumption and continued to decline during the study period (Figure 4). No adverse events were reported or observed during the study due to ingestion of either of the solutions, and none were reported to the clinic staff after the conclusion of the study period. Additionally, relapse of symptoms was not subsequently reported to the clinic staff.

DISCUSSION

In this randomized controlled trial, we demonstrate that compared to ORS alone, supplementation of a novel LiveXtract solution (LifeDrops) significantly decreased resolution time of acute diarrhea and accelerated normalization of stool consistency. Using a cross-over study design, we show that the introduction of ORS+LX is associated with rapid diarrheal resolution, despite differences in induction times between the two cohorts. All patients in the study experienced faster resolution of their diarrhea after receiving ORS+LX, and all soon achieved normalization of stool consistency. The intervention cohort receiving ORS+LX had normalization to BSS $\leq$ 4 stool consistency and frequency by the end of day 1. Similarly, control patients who crossed-over to ORS+LX on day 2 (after receiving ORS+water on day 1) reported comparable efficacy by the end of day 2.

Secondary outcome measures of abdominal pain and defecation urgency also improved for both cohorts upon initiation of ORS+LX by the end of the same day. By the end of the monitoring period on day 2, patients in the control cohort noted a reduction in both adverse symptoms similar to patients in

198 the intervention cohort reported by the end of monitoring on day 1. No adverse events were reported or
199 observed in any patient receiving ORS+LX.
200
201 One limitation of our study is the lack of infectious pathogen identification in subjects' acute diarrheal
202 illness. This study was conducted at a government-funded community health clinic in Managua,
203 Nicaragua following torrential rains and flooding in this region in late 2010. Resource limitations and
204 prioritization of streamlined humanitarian efforts made pathogen identification difficult in the context
205 of a clinical trial, although subjects with evidence of any protozoa by light microscopy were excluded
206 and referred for treatment. Given our hypothesis that the LiveXtract solution maintained the natural
207 antibacterial properties of *Camellia sinensis* and *Punica granatum* within the enteric tract after
208 consumption, we theorize that plant extracts rich in polyphenols have the potential to stimulate innate
209 host immune processes by action of phyto chemicals from natural plant immunity and to antagonize
210 common enteric pathogens responsible for acute bacterial and viral gastroenteritis. Previous literature
211 has identified waterborne enteric pathogens as likely gram negative bacterial species, such
212 enterotoxigenic *Aeromonas, Campylobacter, Salmonella, Shigella,* and enterotoxigenic *Escherichia*
213 *coli*, which all thrive in warm freshwater environments (Burke *et al.*, 1983; Ashbolt 2004), reproduced
214 in the natural elements present in our study.
215
216 Another limitation is that our data represent a snapshot of a narrow study timeframe and one specific
217 geographical location. In an effort to maximize sample size, the cross-over study design could also be
218 viewed as a sub-optimal design, especially in the context of a non-static disease process in acute
219 gastroenteritis. While acknowledging the weaknesses of our study, we also recognize the strength of
220 our study's randomized study design. For a prospective pilot study, we surpassed adequate enrollment
221 numbers to show a clear statistical difference between ORS+LX vs. ORS+water. Among the

222 individual subjects, we showed distinct reproducibility of the treatment effect upon introduction of
223 ORS+LX between individual patients.
224
225 The limited number of pediatric patients who provided data for secondary outcome measures did not
226 permit statistical analyses of the changes in these patients' quality of life. However, the data do show a
227 trend of reducing abdominal pain and defecation urgency with consumption of the polyphenol
228 supplement, which needs to be verified in future clinical outcome studies.
229
230 Preventing and reducing morbidity and mortality from acute diarrheal illnesses causing dehydration is
231 a significant public health concern, and remains an on-going global health initiative. Although the use
232 of ORS to restore intravascular fluid losses remains the standard of care in most clinical scenarios,
233 there are limited clinical alternatives aimed to actively shorten the time of acute diarrheal fluid and
234 electrolyte losses. LiveLeaf LifeDrops solution potentially represents a novel approach to effectively
235 reduce morbidity and mortality from acute diarrhea illnesses in certain situations. In this preliminary
236 study, we report the results of the first prospective clinical trial using this unique supplement to ORS.
237 Published literature in this area includes several negative studies of the addition of rice or non-
238 digestible carbohydrates to ORS (Sarker et al., 2001; El-Mougi et al., 1994; Hoekstra *et al*., 2004;
239 Faruque *et al*., 1997; Khan *et al*., 2005). Further literature review of the efficacy of trace elements such
240 as zinc (Gregorio *et al.,* 2007; CHOICE Study Group 2001) and probiotics (Basu *et al*., 2007; Wadhwa
241 *et al*., 2011) to reduce acute diarrheal disease burden are mixed. The current recommendation of the
242 World Health Organization (WHO) is to provide low osmolarity ORS and zinc supplementation for 10
243 to 14 days (Burke *et al*., 1983), which is associated with reduced time to resolution of diarrhea in
244 several clinical studies (El-Mougi *et al.*, 1994; Gregorio *et al*., 2007; Boran *et al*., 2006; Dutta *et al*.,

2000; Patel, Badhoniya & Dibley 2013), but with times substantially longer than the 3 hours noted in patients given the LiveXtract solution.

Future directions should be aimed at understanding the mechanisms of phytochemicals as potential consumable agents effective in acute infectious gastroenteritis. Elucidation of the molecular basis of the phytochemicals' action on enteric pathogens – through a detailed biochemical pathway – should be pursued, as well as their possible interaction with innate host intestinal immune systems, supported by microbiota analysis. Clinical research efforts should also be directed to test the robustness of our initial efficacy data through reproducibility while subject to contextual study variability.

CONCLUSION

In this randomized, crossover clinical study, pediatric patients with acute diarrhea experienced rapid improvement of stool consistency following ingestion of the LiveXtract solution. Further clinical data are necessary in order to corroborate these results, but the rapid resolution in pediatric patients in this study suggests a well-tolerated, safe, and effective option for the resolution of acute diarrhea syndrome.

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Figure 1. Study design and patient disposition. Patients randomized to the Study Arm were given a mixture of oral rehydration salts (ORS) and LiveXtract (LX) solution (test solution) on day 1 and then a mixture of ORS and water (placebo) on day 2. Patients randomized to the Control Arm were given a mixture of ORS and water on day 1 and then a mixture of ORS and LiveXtract solution on day 2.

Figure 2. Mean time (hours) to resolution of acute diarrhea following consumption of either a mixture of oral rehydration salts (ORS) and LiveXtract (LX) solution (test solution) or a mixture of ORS and water (placebo) on day1 and day 2 of the study. On day 1, the mean times to resolution were significantly different (p=0.002).

Figure 3. Mean ranking of abdominal pain over two days at 30 minute intervals, after consuming either a mixture of oral rehydration salt (ORS) and LiveXtract (LX) solution (study arm) or ORS mixed with water (control arm). Pain was ranked between 0 (no pain) and 10 (worst pain imaginable).

Figure 4. Mean ranking of urgency to defecate over two days at 30 minute intervals, after consuming either a mixture of oral rehydration salt (ORS) and LiveXtract (LX) solution (study arm) or ORS mixed with water (control arm). Urgency was ranked between 0 (none) and 10 (unable to control).

Table 1(on next page)

Serving size of LiveXtract solution administered based upon the weight of the patient.

Table 1. Serving Size of LiveXtract Solution Administered based upon the weight of the patient.

Weight of Patient, kg	Serving Size, mL
10 to 19	3.5
20 to 29	7.0
30 to 39	10.5
40 to 49	14.0
50 to 59	17.5

Table 2(on next page)

Demographics of study population given oral rehydration solution and water (ORS+water) and oral rehydration solution and LiveXtract solution (ORS+LX).

Table 2. Demographics of study population given oral rehydration solution and water (ORS+water) and oral rehydration solution and LiveXtract solution (ORS+LX).

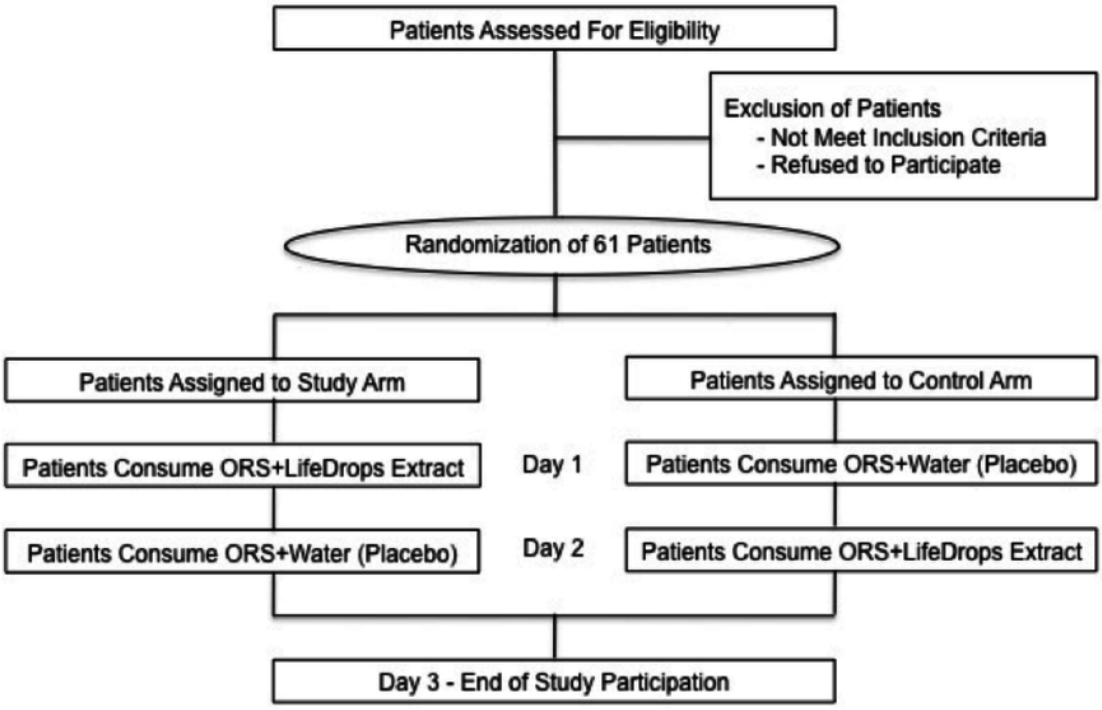
Demographics	Study Arm (n=30) (ORS + LX)	Control Arm (n=31) (ORS + Water)	*P*
Age, mean (SD), years	8 (5.33)	7 (5.53)	0.51[a]
Weight, mean (SD), kg	32 (19.89)	27 (19.32)	0.31[a]
Sex (Male/Female)	13 / 17	18 / 13	0.16 (Study arm)[b] 0.11 (Control arm)[b]

a Student's t-test, significance set at 0.05
b Chi-squared test, significance set at 0.05

1

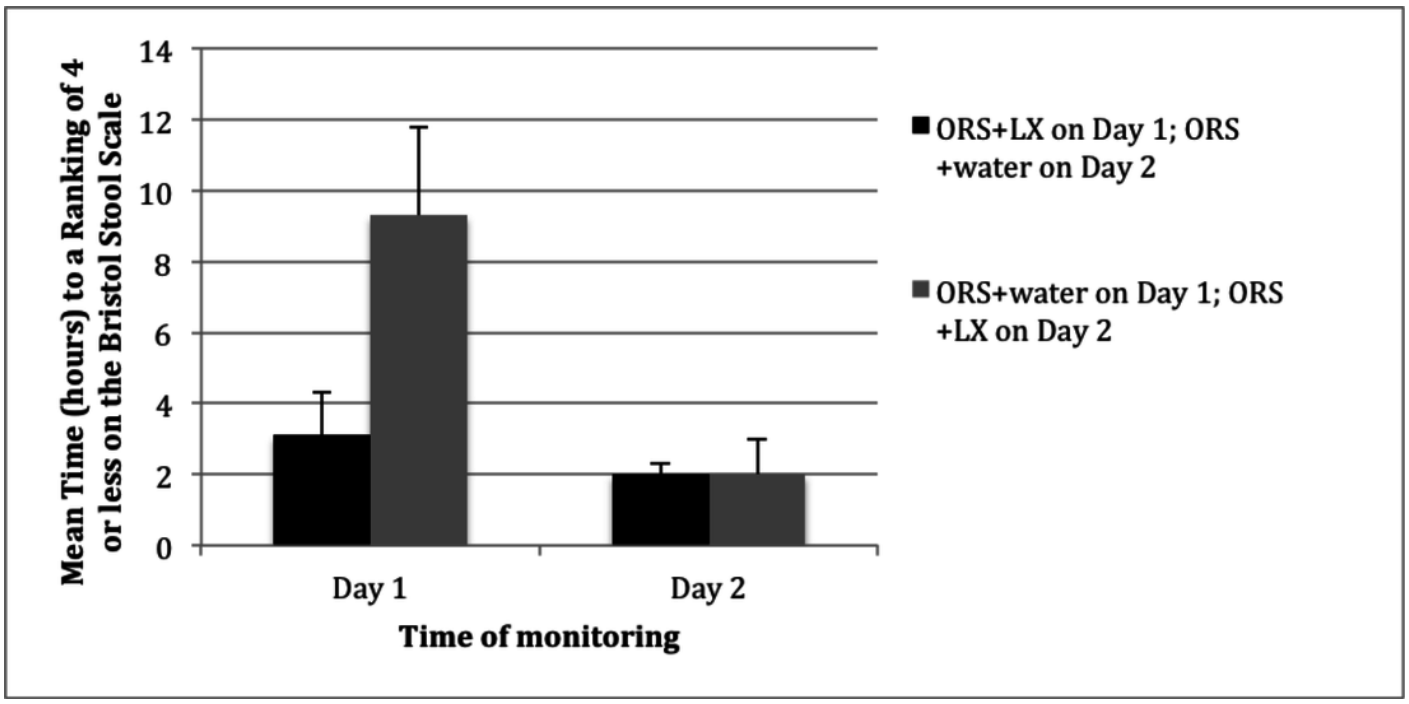
Study design and patient disposition.

Patients randomized to the Study Arm were given a mixture of oral rehydration salts (ORS) and LiveXtract (LX) solution (test solution) on day 1 and then a mixture of ORS and water (placebo) on day 2. Patients randomized to the Control Arm were given a mixture of ORS and water on day 1 and then a mixture of ORS and LiveXtract solution on day 2.



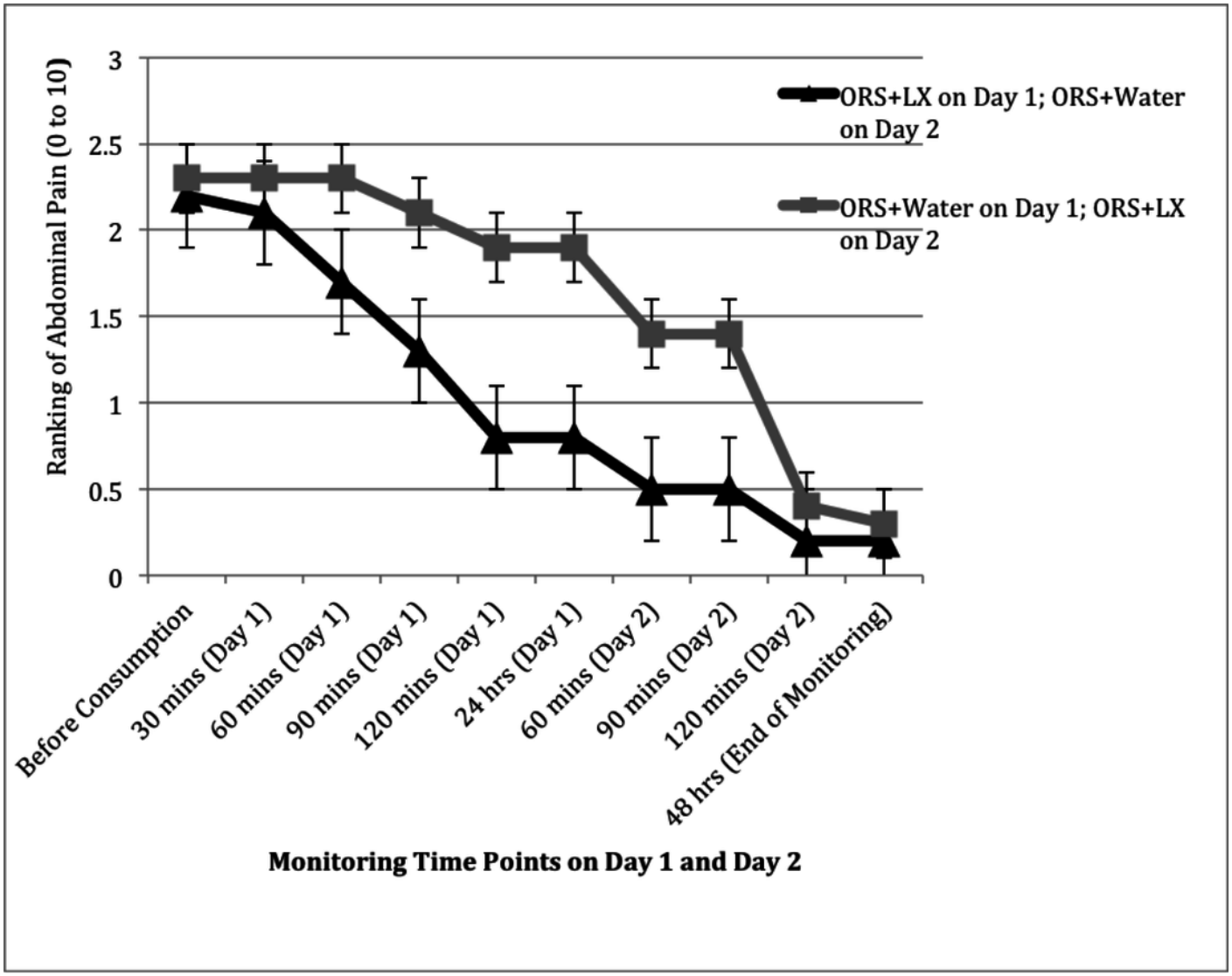
2

Mean time (hours) to resolution of acute diarrhea following consumption of either a mixture of oral rehydration salts (ORS) and LiveXtract (LX) solution (test solution) or a mixture of ORS and water (placebo) on day 1 and day 2 of the study.



3

Mean ranking of abdominal pain over two days at 30 minute intervals, after consuming either a mixture of oral rehydration salt (ORS) and LiveXtract (LX) solution (study arm) or ORS mixed with water (control arm).



4

Mean ranking of urgency to defecate over two days at 30 minute intervals, after consuming either a mixture of oral rehydration salt (ORS) and LiveXtract (LX) solution (study arm) or ORS mixed with water (control arm).