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Original Research Article (Clinical)

A randomized open label efficacy clinical trial of oral guava leaf decoction in patients with acute infectious diarrhoea

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ABSTRACT

Background: Diarrhoea is amongst the first ten causes of death and its treatment faces an increased threat of drug resistance. Previous studies on the guava leaf decoction (GLD) revealed its suitability for use in infectious diarrhoea of unknown etiology.
Objective: The objective of this trial was to establish efficacy, dose and safety of GLD prepared from the Indian *Sardar* variety in adults with acute infectious diarrhoea.
Methods: The current trial was an open efficacy randomized 5-day, parallel group multi-arm interventional study. Amongst 137 adults (18–60 years) suffering with acute diarrhoea, 109 were included (57% females, 43% males). Three doses of GLD (6-leaf, 10-leaf and 14-leaf) were compared with controls receiving oral rehydration solution. Decrease in stool frequency and improvement in consistency were the outcomes measured. The data was analyzed using ANOVA, Tukey's post-hoc test, Kruscal-Wallis test and Chi-Square test where applicable.
Results: The trial showed that the 14-leaf (7.4 g) decoction was the most effective. Administration of the decoction, thrice daily helped the patients regain normalcy in 72 h as opposed to 120 h in controls. Safety of the intervention was reflected by normal levels of haemoglobin, liver and kidney parameters. No adverse events were reported.
Conclusion: The 14 leaves decoction was a safe treatment for adult acute uncomplicated diarrhoea of unknown etiology. Moreover due to component synergy and divergent mechanisms of action, it could possibly combat the generation of drug resistance and destruction of gut microbiota. Hence GLD has the potential for development as a first line treatment for diarrhoea.
Trial registration: Trial was registered with Clinical Trials Registry - India (CTRI registration number: CTRI/2016/07/007095). The trial was retrospectively registered.

1. Background

Gastrointestinal infections such as infectious diarrhoea are amongst the common debilitating infections which affect people of all age groups across the world [1]. The Global Burden of Disease (GBD) Report 2013, listed diarrhoeal diseases as the ninth leading cause of death globally [2]. Every year 1.3 million people succumb to this disease [3]. Whilst diarrhoeal related deaths are common in young children, in survivors it can possibly lead to malnutrition and impaired growth [4]. In adults, while the impact may not be that severe, nutritional deficiencies may arise especially in case of persistent diarrhoea [5]. While public health measures of safe drinking-water, adequate sanitation and hygiene are being implemented [6], there is an urgent need for better treatment that is sustainable, affordable and easily available to combat this disease.

Diarrhoea, as perceived by the general population, is the frequent passing of loose/watery stools. It is either a result of physiological disturbances or an enteric infection. Infectious diarrhoea is caused by various pathogens; the most common enteropathogens are

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rotavirus, *Escherichia coli*, *Shigella* sp., *Vibrio cholerae*, *Salmonella* sp., *Campylobacter jejuni* and *Cryptosporidium.* [7].

As a result of the high mortality rates associated with diarrhoea there is an increase in global attention towards the management of diarrhoea through medications like formulations of oral rehydration solution (ORS) and the development of a suitable vaccine. [6] The response to vaccines, especially in developing countries is not encouraging. [8] Moreover, as multiple pathogens cause diarrhoea, development of a suitable vaccine to combat diarrhoea is challenging. Although ORS has contributed towards reduction in diarrhoeal mortality rates; it is often not able to control the high stool output state. [9] Hence patients resort to antimotility agents which are often contraindicated especially in children [10]. Self-medication and irrational prescription patterns are not only detrimental to gut microbiomes but have led to the emergence of drug resistance [11,12]. An alarming global increase in anti-microbial resistance as seen from recent studies has further necessitated the search for alternatives. Studies reported from Iran stated that resistance of *Shigella* to Cotrimoxazole was 90.24% and in a Kenyan study, 62.5% of diarrhoeal isolates showed multidrug resistance. [13,14] A study from India reported that approximately 70% of childhood diarrhoea was caused by *E. coli* that were multidrug resistant [15]. Another report from Pune, India showed that 88% *V. cholerae* isolates were resistant to furazolidone and 90% to ampicillin [16]. Thus due to the high prevalence of drug resistance there is a need for better and newer drugs. Since the discovery of newer antibiotics would eventually lead to the generation of drug resistance, alternative approaches to treat infectious diarrhoea are required. Medicinal plants which are rich in phytoconstituents can be explored for their divergent mechanisms to fill this niche [17].

A number of plants are used in folklore medicine for treating gastrointestinal ailments across the globe. In a study carried out by the Foundation for Medical Research (FMR), in the Parinche Valley of Pune, India, 28 plants were documented for treating diarrhoea. *Psidium guajava* (guava, leaves) was amongst these cited plants [18] and literature showed that it is widely used as an anti-diarrhoeal worldwide [19]. In India, the use of guava leaves for the treatment of diarrhoea is found in *Ayurveda* and *Unani* literature [20,21]. As per *Ayurveda* guava (*Peruka*, *Paravata*) is a *tridosha nashaka* and useful as *atyagni* [22]. A publication by Central Council for Research in Ayurveda and Siddha, (CCRAS, Government of India) describes the *Ayurvedic* properties of guava 'are as follows' ***Rasa***: *Kashaya, Madhura, Amla*; ***Guna***: *Tikshna, Guru*; ***Veerya***: *Sheeta*; ***Vipaka***: *Madhura*; ***Doshaghnata***: *Vatapittashamaka*; ***Rogaghnata***: *Jeernatisara,* ***Atisara****, Chhardi, Visuchika*; ***Karma***: *Vrishya, Shukrala, Madanashaka, Ruchya, Grahi* which supports its use as an anti-diarrhoeal [23]. In a book on *Unani* medicine '*Khazaain-al-Advia*', a formulation '*Joshanda-e-Amrood*' prepared from guava leaves has been specifically mentioned for treatment of diarrhoea [21].

Studies undertaken at the FMR have revealed that guava leaf decoction (GLD) shows anti-rotaviral, anti-giardial [24] and anti-bacterial activity against *V. cholerae* and *Shigella sp.* [25] Although the decoction did not show bactericidal activity against *E. coli*, it inhibited the colonization and production of labile toxin and also inhibited the IL-8 production [25,26]. The invasion by *S. flexneri* into HEp-2 cells and production of cholera toxin were also affected [25]. Collectively, data from these *in vitro* assays imply that GLD may be effective in controlling infectious diarrhoea caused by a wide spectrum of pathogens. Additionally, since it acts at various stages in the pathogenic process the development of drug resistance with GLD would be minimized. It is probable that the observed efficacy at different levels of pathogenesis is a result of synergistic action of multiple phytoconstituents in GLD. Guava decoction also showed positive results in the *Citrobacter rodentium* mouse model for diarrhoea [27]. The promising results from these *in vitro* and *in vivo* studies was the basis of selecting GLD for the current proof of concept clinical trial. Despite India being largest producer of guava in the world [28], no clinical trial with an Indian variety has been reported. Earlier clinical trials on guava leaves as antidiarrhoeal have been reported from Mexico and China [29–31].

The objective of the present trial was to establish the clinical efficacy of GLD in adult patients with uncomplicated infectious diarrhoea and determine an efficacious dose using leaves from the common Indian variety '*Sardar*'. A concurrent objective was to establish safety of GLD concentrations used in the trial. The anti-diarrhoeal effect of the tested intervention was assessed based on its capacity to reduce stool frequency and improve consistency. Though incidence of diarrhoea is more prevalent in the paediatric population than in adults [32], it was considered safe to initiate the proof of concept study in the adult population especially since the dose needed to be ascertained.

2. Methods

The clinical trial was conceptualized by TB (corresponding author) from FMR in Mumbai following extensive *in vitro* and *in vivo* studies on the effect of guava decoction on diarrhoeal pathogens. As FMR could not conduct the clinical trial at their site due to lack of clinical facilities, Medanta, The Medicity, Gurugram was identified as a collaborator for undertaking of the trial. GGK and SK, the co-authors from the hospital jointly treated the patient and supervised the trial at the site. The guava leaves required for the trial were sourced and processed by FMR and then sent to Medanta hospital for the trial. Trial was approved by the Ethics Committees of both FMR (IEC/MP/01/2015) and the Medanta hospital (553/2015).

2.1. Trial site

The clinical trial was conducted between July 2016 to March 2018 at Department of Integrative Medicine, at Medanta hospital. Patients visiting the Out-Patient Department (OPD) of the hospital were enrolled in the trial.

2.2. Study design

The trial was a 5 day, randomized, parallel group, multiple arm interventional study having an intended patient allocation ratio of 1:1:1:1 with an objective to evaluate the clinical efficacy of guava leaf decoction in treatment of acute adult diarrhoea. The study adheres to the CONSORT guidelines.

2.3. Definition of diarrhoea

Diarrhoea was defined as per World Health Organization (WHO) guidelines, as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Frequent passing of formed stools was not considered as diarrhoea [33].

2.4. Participant enrolment

Diarrhoeal patients were enrolled in the study based on their clinical symptoms. Identification of the causative organism of diarrhoea through microbial analysis of the stool sample was not undertaken in this study. However microscopic stool examination for occult blood, presence of red blood cells (RBCs), pus cells and cyst/ova was undertaken. Motility (for *V. cholerae*) using the hanging drop method was also done. These parameters were monitored on screening day, day 3 of treatment and on completion of treatment.

Enrolled participants were allocated to either one of the four groups (3 doses of GLD or control) to achieve block randomization based on a computer-generated randomisation list. The allocation was done under the control of the study statistician who revealed allocation via serially numbered opaque sealed envelopes one by one. A clinical research associate (CRA) then examined the allocation envelope and accordingly prepared the decoction if required, as per the allotted group.

2.5. Inclusion criteria

Adults (age: 18–60 years) presenting with acute infectious diarrhoea of >1 day and <14 days duration. Diarrhoea was defined as per the WHO definition - ≥3 loose stools per day OR liquid stools OR more than normal for that particular person. Its infectious etiology was presumptive based on presence of fever, nausea/vomiting or abdominal pain/cramps.

Patients with uncomplicated and controlled hypertension and diabetes were not excluded.

2.6. Exclusion criteria

Patients with the following conditions were excluded from the study

1) Non-ambulatory patients.
2) Suffering from diarrhoea as a result of antibiotic treatment, food allergies, ingestion of laxatives (72hrs prior to reporting).
3) Presenting with symptoms of irritable bowel syndrome.
4) Those who were already on anti-diarrhoeal treatment.
5) With chronic kidney, liver or cardiac problems or with gastrointestinal complications (as evident from the sonography done at screening).
6) Patients presenting with visible blood in stools.
7) Pregnant/lactating/menstruating women.
8) Candidates showing symptoms of gross malnourishment, having fever >101 °F at the time of enrolment or with blood pressure (Blood Pressure) levels of <90/60 mm of Hg as a result of moderate or severe dehydration.

2.7. Consent

A written consent was obtained from patients in a predesigned consent form, following an explanation about the type of study, translated into local language (*Hindi*). The interaction with the patient while taking consent was audio/video recorded. One hundred and twenty-four patients were counseled of which 109 patients were finally enrolled.

2.8. Treatment groups

All patients who suffered from dehydration were advised ORS irrespective of their treatment group.

Since only a few clinical trials have been undertaken with GLD, information with respect to dosage is limited [34]. Ethnobotanical data on the use of guava decoction though available, does not specify the dose used. One report mentioned the use of a handful of leaves for the preparation of the decoction [35]. The number of leaves in a handful averaged 6 leaves. Hence for this proof of concept clinical trial, for which determination of an efficacious dose was one of the objectives, decoction prepared from 6 leaves was considered as the median dose and one dose lower (2 leaves) and one dose higher (10 leaves) was initially used for the trial. The groups were labelled as Group I- decoction made from 2 leaves, similarly Group II- 6 leaves and Group III- 10 leaves. The dose given to each patient was randomized as stated in section 2.4 and not dependent on the severity of diarrhoea.

Patients from the intervention Groups who failed to recover post treatment, were to be treated using standard treatment for diarrhoea. All patients allocated to the intervention Groups recovered from diarrhoea and hence no patient cross over was reported in this trial.

2.9. Amendment in protocol

Towards the end of February 2017, after 30 patients were enrolled in the trial; 9 of which were enrolled in Group I (2 leaves), it was noted that the patients in this group had no significant improvement in symptoms when compared with the control. Although no patient remained uncured, the time required for reaching normalcy was similar to the control group. Hence in March 2017 the trial protocol was amended. The group initially being treated with 2 leaves was terminated. Fresh consequent patients who got enrolled under the title of Group I were given decoction prepared from 14 leaves instead of 2 leaves. This 14-leaf group has been referred to as Group IV. Another amendment was made in January 2018 towards recruiting further patients in the 14-leaf group only to allow all groups to have equal number of patients by the end of the trial (March 2018). The two amendments were approved by the Ethics Committee of both the institutes and the Project Advisory Committee. These were also conveyed to the funding agency.

2.10. Parameters assessed

2.10.1. At screening

At the time of screening a predesigned patient proforma was filled by the study CRA. Besides basic information such as age and gender, the proforma covered details on number of days with diarrhoea, number of stools in previous 24 h, the consistency of stools graded on a scale 1 to 4 (1: watery, 2: liquid, 3: semisolid, 4: solid/normal), presence of blood/mucous in the stools, presence of other common symptoms such as nausea, fever, vomiting, abdominal pain (graded on a scale of 1–10) and abdominal distension. The following investigations were also undertaken- (a) Routine stool, (b) blood culture, (c) complete blood count (CBC), (d) Bilirubin, SGPT (Serum Glutamic Pyruvic Transaminase) and SGOT (Serum Glutamic Oxaloacetic Transaminase) as indicators of liver function (e) Kidney function as measured by the serum creatinine and blood urea. Routine microscopic stool examination included checking for presence of blood, mucus, cysts and motile bacteria (indicative of *Vibrio*) in stools. Blood culture was also done to rule out typhoid.

2.10.2. Day 3

Apart from the parameters such as frequency of stools, consistency of stools, abdominal pain and distension, episodes of vomiting and reporting of fever, additional routine microscopic examination of stools was done on day 3.

2.10.3. On completion of treatment

The investigations undertaken on screening day were repeated. These included (a) Routine stool microscopy, (b) blood culture, (c) CBC, (d) Liver function tests (Bilirubin, SGPT and SGOT) (e) Kidney function tests (serum creatinine, blood urea), (f) Routine microscopic stool examination.

Detailed criteria were framed towards rescue medication, discontinuation of patients from trial and a proforma was also

designed to record and report any serious adverse event due to intake of guava decoction.

2.11. Outcome measures

The parameters considered indicative of efficacy were reduction in frequency of stools, improvement in consistency of stools, reduced nausea, abdominal pain and episodes of vomiting. These parameters were recorded daily during the course of treatment (5 days) and up to day 7. Though a frequency of ≤ 2 is considered to be normal, in the present trial a patient was considered to have attained normalcy if the stool frequency was 1.

2.12. Criteria for discontinuing patient from the trial

Patients were to be discontinued from treatment, who

(i) reported with body temperature more than 101 ° F,
(ii) had more than twice the stool frequency
(iii) had twice the episodes of vomiting as compared to earlier day
(iv) had double the score for abdominal pain as on previous day or
(v) had severe constipation (no stool for 48 h).

2.13. Details of raw material (guava leaf) used in the trial

Mature guava leaves of *Sardar* variety used in the trial were collected as a single batch from Shirwal, Satara district, Maharashtra during September 2014. Dr. P. Tetali, an ethnobotanist authenticated the leaves and a voucher specimen was deposited at Naoroji Godrej Centre for Plant Research (NGCPR) under herbarium number NGCPR 712. The leaves were collected, washed clean, shade dried at the source and then transported to FMR in Mumbai and stored at 25 °C.

Two hundred grams of dried leaves were sent to National Agricultural and Food Analysis and Research Institute (NAFARI), Pune, Maharashtra to screen for presence of aflatoxins (B1, B2, G1, G2), heavy metals (copper, zinc, lead, cadmium, arsenic, tin, mercury) and also to determine the microbial load. The methods used at NAFARI were High Performance Liquid Chromatography (HPLC) and Atomic Absorption Spectroscopy (AAS) for aflatoxins and heavy methods respectively. The references cited in their report were Association of Analytical communities (AOAC), 2012 and Indian Standards (IS), 2012.

Leaves were coarse ground, distributed into different pouches based on the different doses for each treatment Group and couriered to the hospital partner. These were then stored at 25 °C in the pharmacy of the hospital under the supervision of the pharmacist.

2.14. Preparation of decoction

A single batch of mature guava leaves was used for the entire trial. Decoction was prepared as per *Ayurvedic* text [36] and under supervision of an *Ayurvedic* physician, GGK, the co-author from Medanta hospital. To minimize variability between individual decoction preparations, the fresh decoction was prepared every day under the same conditions (same vessel, burner) only by the trained CRA and dispensed to the patient.

The average weight of each guava leaf was estimated at 0.53 ± 0.07 g. Hence the weights of guava leaf powder for the groups was Group I- 1.6 gm, Group II- 3.2 g, Group III- 5.3 g and Group IV- 7.4 g. The decoction was prepared by boiling appropriate amount of leaf powder in 120 ml of water on an open flame until the volume was reduced to 30 ml. This was then divided into 3 doses of 10 ml each for dispensing. The varying amounts of guava leaf powder in a fix amount of water reflected the increasing concentration of the decoction.

Each patient received the freshly prepared decoction every day in three divided doses (10 ml) for a total duration of 5 days. Control group patients did not receive the decoction but only standard care treatment comprising of ORS as required for rehydration.

As the decoction was freshly prepared for each patient and was to be taken three times in a day, it was important to know if there was any degradation over this period of time. Hence the stability of a representative decoction was checked over a 24-h period using High Performance Liquid Chromatography (HPLC). Quercetin, a major flavonoid present in leaves [37], was used as a reference standard. HPLC was carried out at the Institute of Chemical Technology, Mumbai.

2.15. Ethical Approval and trial registration

Trial was approved by the ethics committee of both FMR (IEC/MP/01/2015) and the Medanta hospital (MICR:553/2015)

The trial was registered with the Clinical Trials Registry - India (CTRI registration number: CTRI/2016/07/007095). The trial was retrospectively registered. Two amendments were made in the protocol in March 2017 and January 2018.

3. Statistical analysis

Twenty-five patients were proposed to be enrolled in each group and this number was decided empirically since data was not available from which statistical power and sample size could be estimated. Data for 109 patients who completed the trial was analyzed using SPSS v19 (SPSS, Inc., Chicago, IL, USA). ANOVA with Tukey's post hoc test was used to analyze the parametric data and for non-parametric data Kruscal-Wallis test was used (consistency of stools and abdominal pain data). Results with $P \leq 0.05$ were considered significant. Proportions (patients reaching normal stool frequency) were analyzed using Chi-Square test.

Due to the protocol amendment as stated in Section 2.9, in the results, the 2 leaf group has been included only in parameters which did not involve any statistical analysis. These include the demographic details of patients on screening day (Table 1 - Mean ± S D values for all parameter at screening day), and Pharmacological effect (Fig. 3).

4. Results

The results of the tests carried out at NAFARI, on the guava leaves used for the trial, revealed that aflatoxins and heavy metals were within permissible limits and the total microbial count was found to be 2.3×10^4 CFU/g with absence of *E. coli* **(Supplementary Data)**.

Stability of the decoction studied over a period of 24 h using HPLC indicated that the fingerprint did not show any significant deterioration and quercetin reduced marginally by 0.05% after 24 h from 19.28 μg per ml to 19.27 μg per ml (Fig. 1).

Patient enrollment: Patients visiting the OPD of the hospital between July 2016 and March 2018 for treatment of diarrhoea and who gave consent to participate in the trial were enrolled for the study. One hundred and thirty-seven patients approached the hospital for diarrhoeal treatment, 13 of whom were excluded based on the exclusion criteria as they had either a) taken antibiotics prior to approaching the OPD of the hospital (n = 6), b) had visible blood in stools (n = 2), c) were lactating women (n = 2), d) had taken laxatives (n = 2) or e) had fever ≥101 F (n = 1). Fifteen patients refused to participate in the study due to the following reasons: a) travelling

Table 1
Demographic details of the study.*

	Control (n = 25)	Group I (n = 9)	Group II (n = 25)	Group III (n = 25)	Group IV (n = 25)
Gender					
Male	11 (44%)	7 (78%)	15 (60%)	9 (36%)	5 (20%)
Female	14 (56%)	2 (22%)	10 (40%)	16 (64%)	20 (80%)
Age	31 ± 10	33 ± 8	30 ± 9	27 ± 6	29 ± 6
Number of days suffering with diarrhoea prior to enrollment	3 ± 2	5 ± 4	3 ± 2	3 ± 1	3 ± 2
Frequency of stools	6.16 ± 1.54	7 ± 2.24	5.84 ± 2.04	5.24 ± 0.97	5.24 ± 1.45
Consistency of stools	1.8 ± 0.71	2.22 ± 0.441	1.8 ± 0.65	1.8 ± 0.71	1.36 ± 0.49
Abdominal pain	2 ± 2.47	1.44 ± 1.51	0.96 ± 1.65	1.12 ± 1.9	2.36 ± 2.5
Episodes of Vomiting	0.36 ± 0.91	0	0.56 ± 1.6	0.08 ± 0.4	0.04 ± 0.2
Total WBC count*(10^3/µl)*	7.89 ± 2.86	7.53 ± 1.79	8.78 ± 5.67	8.41 ± 2.37	7.87 ± 2.02
Bilirubin *(mg/dL)*	0.82 ± 0.48	0.90 ± 0.41	0.83 ± 0.33	0.77 ± 0.30	0.64 ± 0.5
SGOT *(U/L)*	29.92 ± 10.93	32.56 ± 14.49	30 ± 10.5	29.32 ± 11.68	26.20 ± 7.17
SGPT *(U/L)*	40.64 ± 20.6	38.33 ± 16.10	35.88 ± 11.41	39.04 ± 26.43	39.20 ± 20.54
Serum creatinine *(mg/dL)*	0.72 ± 0.19	0.68 ± 0.14	0.75 ± 0.16	0.66 ± 0.16	0.65 ± 0.11
Blood urea *(mg/dL)*	20.04 ± 5.91	23.22 ± 6.92	21.44 ± 6.95	21.40 ± 8.34	19.48 ± 5.84
Haemoglobin *(g/dL)*	13.15 ± 1.81	14.26 ± 1.26	13.61 ± 2.26	13.14 ± 2.13	12.21 ± 1.92

*Mean ± sd values for all parameters on the screening day.

out of town (n = 4), b) scared to enroll in a clinical trial (n = 7), c) not willing to take *Ayurvedic* medication (n = 4). At the end of the trial in March 2018, a total of 109 patients therefore completed the study. Nine patients were assigned to Group-I (2-leaf), and 25 each to Control, Group -II, Group III, and Group-IV (6-leaf, 10-leaf, and 14-leaf groups respectively). There was no patient loss due to: loss to follow-up or discontinuation of intervention by the patient in any group. Hence data from 109 patients was used for the final analysis.

The enrollment, allocation, follow-up and analysis scenario of the trial is depicted through the CONSORT flow diagram (Fig. 2).

4.1. Demographic details and baseline data

Out of the 109 patients enrolled in the study 62 were females (56.88%) and 47 were males (43.12%). Data related to the age, number of days suffering with diarrhoea prior to enrollment, frequency of stools, consistency of stools, intensity of abdominal pain and episodes of vomiting on the screening day is reported in Table 1. Additionally, the total WBC count, haemoglobin, liver function parameters and kidney function parameters are also included in this table.

Statistical analysis comparing the different groups at baseline showed that there was no significant difference between them with respect to symptoms viz. number of days suffering with diarrhoea prior to enrollment, frequency of stools, consistency of stools, abdominal pain, episodes of vomiting, total WBC count, haemoglobin, liver function parameters and kidney function parameters.

The patients enrolled in the study suffered for 1–10 days with diarrhoea prior to enrollment. At the time of enrollment, 42

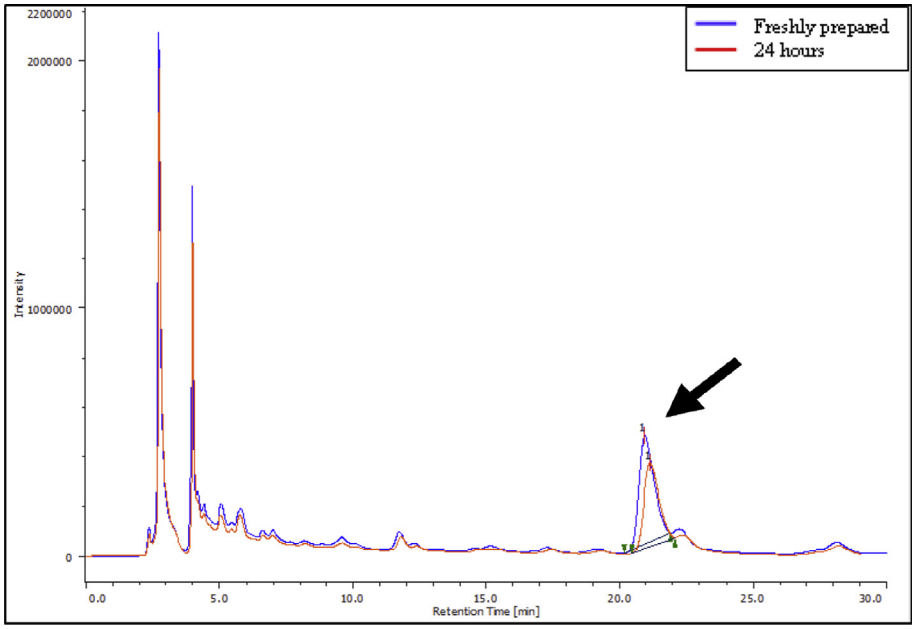

Fig. 1. Overlap of HPLC chromatograms for decoction recorded at 0 time and 24 h. → Quercetin peak.

patients had abdominal pain and 16 patients showed signs of abdominal distension, 4 patients had mucus in stools, 10 patients had episodes of vomiting and 18 patients complained of nausea. Some of the patients experienced more than one of the above symptoms.

4.2. Intervention outcome

4.2.1. Frequency of stools

Control as well as Groups II, III and IV showed significantly decreased stool frequency at 24 h ($p = 0$) as compared to the enrollment day. Tukey's post hoc test indicated that Group III ($p = 0.021$) and Group IV ($p = 0.009$) significantly differed from the control. Time required for the mean frequency of stools to reach normalcy was the least in Group IV (72 h) and maximum for the control (120 h) (Table 2).

Additionally, the proportion of patients reaching normal stool frequency in the various Groups was computed. It was found that the proportion of patients reaching frequency of 2 at 48 h was significantly more ($P = 0.001$; Pearson's Chi-Square value = 11.538) than those in the Control Group. Even at 72 h, the proportion of patients reaching normalcy (frequency = 1) was significantly more ($P = 0.005$; Pearson's Chi-Square value = 8.013) in Group IV than those in the control. The percentage of patients in the Control, Group II and Group III were 32, 40 and 44 respectively. Thus, Group IV was most effective in reducing stool frequency and reaching normalcy.

4.2.2. Consistency of stools

Consistency of stools was graded on a scale of 0–4 and improvement in consistency was indicated by an increase in the score. Significant improvement in consistency was seen by 48 h in all groups, however normal consistency (grade 4) was reached by 72 h for group IV as opposed to control, group II and group III where at least 96 h were required (Table 2).

4.2.3. Intensity of abdominal pain

Patients in the Group IV showed significant improvement in abdominal pain by 48 h. No significant improvement was seen in groups II and III. However, the Control Group also showed significant improvement at 48 h (Table 2).

4.2.4. Episodes of vomiting

Overall only 10 patients complained of vomiting and therefore statistics could not be computed. Relief from vomiting for Control ($n = 3$), Group III ($n = 1$) and Group IV ($n = 2$) was achieved by 24 h whereas Group II ($n = 4$) required 48 h for complete cessation (Table 2).

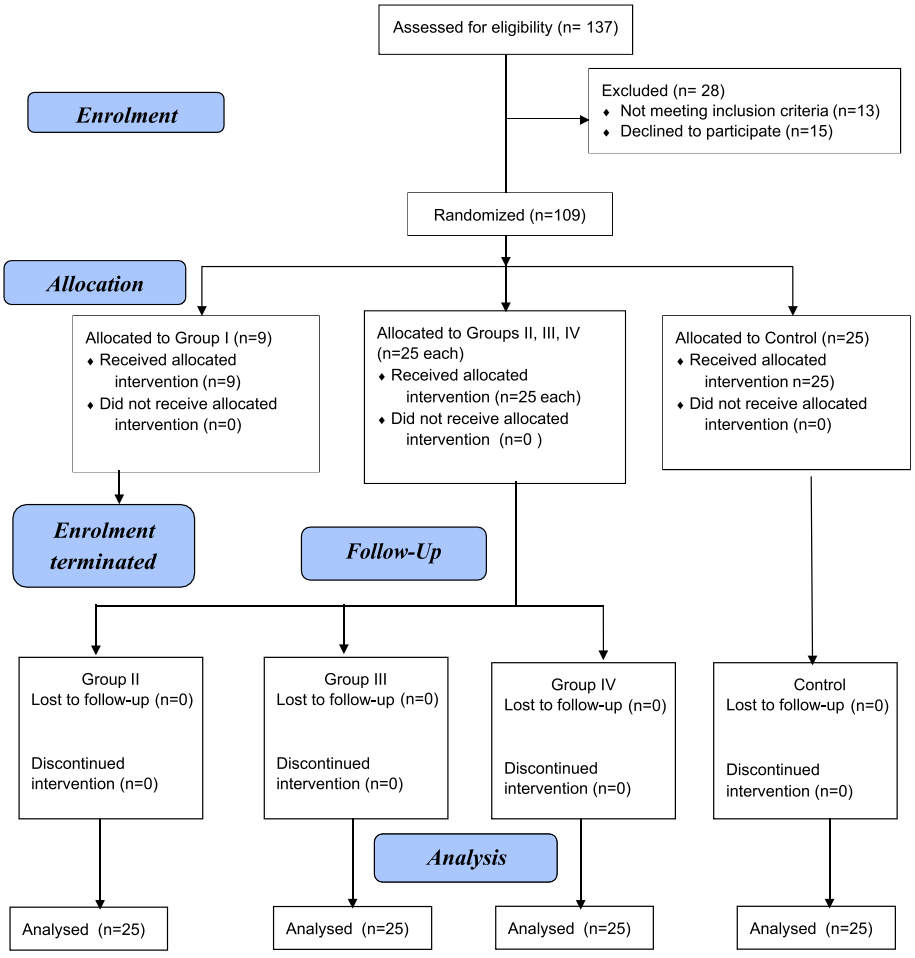

Fig. 2. CONSORT representation of study design.

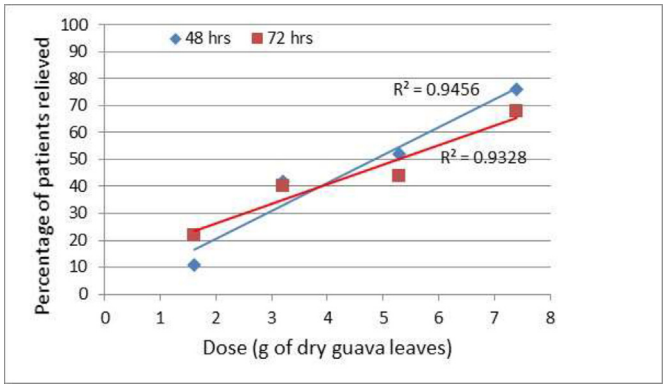

Fig. 3. Dose response curve when normalcy was reached at 48 h with stool frequency of 2 and at 72 h with stool frequency of 1.

4.3. Pharmacological effect

The nature of the dose response curve signifies a pharmacological effect [38]. A dose response curve was plotted to ascertain the pharmacological effect of the decoction. It was seen that the number of patients reaching normalcy was linearly proportional to the dose administered (Fig. 3) indicating a significant pharmacological response.

4.4. Safety

Since guava decoction has been reported to affect gastric motility [39–41], prior to enrollment of patients in the trial, 5 healthy volunteers were administered 10 leaves guava decoction for a period of 5 days to check for any intervention related side effects (data not shown). None of the 5 patients complained of constipation. Since course of treatment of guava leaf decoction is of 5 days in diarrhoeal patients the safety assessment was also carried out with a 5 day regimen.

Haemoglobin, liver function and kidney function parameters did not significantly change over the period of treatment for all the groups including the Control Group (Table 3).

4.5. Adverse events & treatment compliance

No trial related adverse events were reported. Six patients completed the treatment but did not come for the follow-up testing on completion of treatment (Day 7). These patients were followed up telephonically regarding their symptoms.

5. Discussion

Although better sanitation, hygiene and improved quality of drinking water have contributed towards the decrease in incidence of infectious diseases around the world, the prevalence of diarrhoea has not reduced drastically [42]. It still remains the second cause of death after lower respiratory infections in low income countries [43]. Apart from its high prevalence in the adult population, it is also the most common cause of death in children globally including India [3]. Moreover, the frequent use of antibiotics in children can also lead to diseases like diabetes and obesity later in life due to early disruption of gut microbiota [44]. In adults too, though the impact may not be very severe, diarrhoea can substantially affect the quality of life especially in chronic cases [45]. Hence alternative treatment options need to be explored. However, amongst the various anti-diarrhoeal herbal remedies only a few of them have been subjected to formal clinical trials [46].

The current trial was undertaken using guava leaves from *Sardar* variety, which is one of the five most common Indian varieties [47]. The study was a 5 day, randomized, parallel group, multiple arm interventional trial conducted to evaluate the clinical efficacy of guava leaf decoction in treatment of diarrhoea. This trial, conducted on 109 patients suffering from uncomplicated diarrhoea revealed that treatment with guava significantly improved their condition with respect to stool frequency and consistency as compared to baseline. The reduction in frequency of stools and improvement in the consistency was observed within the treatment period of 5 days. Treatment with guava decoction at different doses (6 leaves, 10 leaves, 14 leaves) showed that the mean stool frequency of 1 and consistency of 4 was attained much faster in the 14 leaves group (7.4 g) as compared to the other groups. This was reached within 72 h from the start of treatment. Use of guava leaf decoction was found to be safe over the period of treatment as it did not significantly affect the haemoglobin levels, liver and kidney functions. The outcomes of this trial with respect to the improvement in consistency and decrease in frequency of stools are similar to earlier clinical trials conducted in Mexico and China, using guava leaf powder, tincture or extracts for treating adult acute, simple diarrhoea and infantile rotavirus related diarrhoea [29–31].

Thus guava which is widely grown in India and in many other developing countries can be used as an easily available and economical alternative to current day treatment especially in these regions.

Ayurvedic treatment is often based on maintaining homeostasis rather than any aggressive single targeted effect. This is exemplified by the multiple properties of guava leaves such as *atisara*, *kashaya*, *grahi* and our earlier studies that demonstrated that (a) GLD is not bactericidal against *E. coli*; however it prevented bacterial colonization to gut and reduced toxin production/binding, (b) GLD was cidal against *Shigella* at 1%, but it prevented bacterial invasion into HEp-2 cells at a lower dose of 0.1% (c) GLD did not kill rotavirus directly but viral entry into the host cell was inhibited [25]. Additionally studies carried out in normal individuals at the beginning of this trial

Table 2
Mean of parameters at screening, day of significance and at time when normalcy was achieved.

	Control (n = 25)	Group II (n = 25)	Group III (n = 25)	Group IV (n = 25)
Frequency of Stools (n = 100)				
On Screening Day	6.16	5.84	5.24	5.24
At 24 h	4.84*	4.08*	3.64*	3.52*
At normalcy	1.2	1.46	1.28	1.28
(Time when normalcy achieved)	(120 h)	(96 h)	(96 h)	(72 h)
Consistency (n = 100)				
On Screening Day	1.8	1.8	1.8	1.36
At 24 h	2.16*	2.32*	2.32	2.36*
At normalcy	3.88	3.88	3.88	3.8
(Time when normalcy achieved)	(96 h)	(120 h)	(96 h)	(72 h)
Abdominal pain (n = 42)				
On Screening Day	2	0.96	1.12	2.36
At 24 h	0.64	0.6	0.28	0.8
At normalcy	0	0.24	0.28	0
(Time when normalcy achieved)	(48 h)	(48 h)	(24 h)	(48 h)
Episodes of Vomiting (n = 10)				
On Screening Day	0.36	0.56	0.08	0.04
At 24 h	0	0.4	0	0
At normalcy	0	0	0	0
(Time when normalcy achieved)	(24 h)	(48 h)	(24 h)	(24 h)

P value < 0.05.

Table 3
Mean ± SD of Liver function and kidney function parameters.

	Control (n = 25)	Group II (n = 25)	Group III (n = 25)	Group IV (n = 25)
SGPT (Normal range:21–72 U/L)				
Screening Day	40.64 ± 20.60	35.88 ± 11.41	39.04 ± 26.43	39.2 ± 20.54
On completion of treatment	36.28 ± 11.14	37 ± 9.98	39.18 ± 26.86	35.52 ± 15.27
SGOT (Normal range: 12–59 U/L)				
Screening Day	29.92 ± 10.93	30 ± 10.50	29.32 ± 11.68	26.2 ± 7.17
On completion of treatment	28.04 ± 5.69	29.46 ± 7.44	29.91 ± 12.41	23.48 ± 4.98
*Bilirubin (Normal range: 0.2-1.*3 mg/dL*)*				
Screening Day	0.82 ± 0.48	0.83 ± 0.33	0.77 ± 0.30	0.64 ± 0.50
On completion of treatment	0.74 ± 0.46	0.67 ± 0.34	0.68 ± 0.20	0.48 ± 0.26
*Serum Creatinine (Normal range: 0.8-1.*5 mg/dL*)*				
Screening Day	0.72 ± 0.19	0.75 ± 0.16	0.66 ± 0.16	0.65 ± 0.11
On completion of treatment	0.73 ± 0.19	0.73 ± 0.18	0.61 ± 0.18	0.63 ± 0.07
*Blood Urea (Normal range: 19–*43 mg/dL*)*				
Screening Day	20.04 ± 5.91	21.44 ± 6.95	21.4 ± 8.34	19.48 ± 5.84
On completion of treatment	20.4 ± 5.22	21.25 ± 5.50	20.86 ± 8.76	19 ± 5.65
*Haemoglobin (Normal range: 12–*15 g/dL *(F), 13-*17 g/dL *(M)*				
Screening Day	13.15 ± 1.81	13.61 ± 2.26	13.14 ± 2.13	12.21 ± 1.92
On completion of treatment	12.85 ± 1.74	13.45 ± 2.12	12.67 ± 1.70	11.810 ± 1.66

indicated that treatment with GLD does not lead to constipation even though it is reported to have anti motility activity.

The current Standard of Care treatment for diarrhoea involves administration of ORS which helps rehydration but does not reduce the frequency of stools [9]. Therefore, many practitioners and even patients prefer antibiotics or anti-motility agents. Though the antimotility agents give symptomatic relief, there are increased chances of the patient developing severe constipation leading to conditions like the toxic colon [48]. Loperamide, a common anti-motility agent has been reported to cause constipation [10].

Data from literature as well studies undertaken by FMR have indicated that guava leaves can be used as an alternative to treat infectious diarrhoea of varied etiologies; bacterial, viral and protozoal [24,25,49]. Studies have also documented its use in physiological diarrhoea [50,51]. The influence of guava over the gut microflora has also been reported [52]. Thus, its mechanism in treating infectious diarrhoea could also be a combined effect of its anti-infective and anti-virulent activity supported by its positive effect on the microflora [25,52–54]. Hence a guava-based formulation could be used in conjunction with ORS as a first line treatment to combat diarrhoea.

5.1. Trial limitations

Although diarrhoea is more prevalent in the paediatric group as compared to adults and the mortality in children is higher, the current trial was a proof of concept study and hence had to be undertaken in adults for the purpose of assuring safety of GLD and also determine an efficacious dose. Secondly the trial was based on the *in vitro* and *in vivo* results which suggested the usefulness of GLD in treating diarrhoea of varied etiology. Since bacteriological examination of the stools was not undertaken at screening to identify causative organism, the laboratory results could not be verified clinically from the patients in this trial.

6. Conclusion

A strong case exists for the development of GLD as an anti-diarrhoeal in infectious and physiological diarrhoea. The results of this trial confirm that 14 leaves guava (7.4 g) decoction, is a safe treatment for acute uncomplicated diarrhoea of unknown etiology in the adult population. It does not cause constipation, which is a side effect of some of the commonly used anti-motility agents. The dual effect of guava decoction on the gastric motility as well as against pathogens justifies exploration of its use in physiological and infectious diarrhoea including that are caused by drug resistant bacteria. Therefore, the development of a guava decoction-based formulation could be a promising anti-diarrhoeal remedy. This formulation being phytochemically complex consisting of multiple components as opposed to the currently used anti-diarrhoeals which are single/dual drug preparations would probably minimize the threat of rapid emergence of drug resistance. Another advantage may be the restoration of homeostasis related to the gut microbiota.

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Conflict of interest

Dr. G Geetha Krishnan listed as the second author is on the Editorial Board of the Journal. He was not involved in the review or editorial process of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2020.04.001.

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