

Psidium guajava leaf extract prevents intestinal colonization of *Citrobacter rodentium* in the mouse model

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ABSTRACT

Diarrheal diseases are the second highest cause of mortality of children under 5 years worldwide. There is a continuous search for developing a cost-effective treatment for diarrhea as the present ones are facing challenges. Medicinal plants can be explored further as an alternative treatment for diarrhea. *Psidium guajava* leaves have been used as an anti-diarrheal globally. *Citrobacter rodentium*, a common mouse pathogen, is known to mimic the pathogenicity of enteropathogenic and enterohemorrhagic *E. coli*. It can thus present an effective model to study infectious diarrhea. In the present study, the *P. guajava* leaf extract was tested for its efficacy in treating infectious diarrhea using a *C. rodentium* mouse model. The mice in the test group (treated with *P. guajava* leaf extract) showed quicker clearance of infection as compared with the control group. The bacterial load in the fecal sample of the mice in the test group was high on Day 4 as compared with that in the control group, suggesting a flush out of the bacteria. In the test group, 6/7 (85.71%) mice showed clearance of infection by Day 19. The control group continued to show infection till Day 29. *P. guajava* leaf extract thus has the potential for use in the treatment of infectious diarrhea.

Key words: Antidiarrheal, *C. rodentium*, *P. guajava*

INTRODUCTION

Of the 58% of all deaths in children aged 5–14 years due to infectious diseases in India, infectious diarrhea accounts for nearly 18%.^[1] In young children, it can lead to dehydration and death and in survivors, to impaired growth and malnutrition. The present treatment of diarrhea has been associated with a number of shortcomings such as patient dissatisfaction with the use of oral rehydration therapy (ORT),^[2] emergence of drug resistance due to

excessive use of antibiotics,^[3] non-feasibility of developing a vaccine for the wide spectrum of pathogens involved in a diarrheal episode,^[4] etc., These factors led to the inclusion of traditional medicines along with the conventional treatment in the Diarrhea Disease Control Program by the World Health Organization (WHO). Thus, an important niche lies in developing alternative cost-effective approaches for the prevention of diarrhea, one of them being screening of medicinal plants.

India harbors a rich heritage of medicinal plants, with over 400 known to possess anti-diarrheal properties. The use of *Psidium guajava* (guava) leaves in the treatment of diarrhea has been cited in the Ayurveda^[5] and Unani^[6] literature. Global use of guava in traditional medicine for gastrointestinal problems has also been reported.^[7] Several *in vitro* studies have demonstrated the efficacy of guava leaves against intestinal pathogens.^[8-10] However, the *in vivo* efficacy of guava leaves against infectious diarrhea still needs to be explored.

While there have been substantial advances in the understanding of the molecular pathogenesis of enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC), infecting laboratory mice with these organisms still poses a challenge. Because of this

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limitation, several studies have found the murine bacterial pathogen, *Citrobacter rodentium*, to offer a relevant and robust *in vivo* model to analyze infectious diarrhea.^[11] Mice infected with *C. rodentium* develop mild diarrhea, attaching/effacing (A/E) lesions, pedestal-like projections and colonic hyperplasia, making this system an excellent model to study EPEC and EHEC infection of the human gut.

Pre-clinical *in vivo* biological screening is important as some compounds that show activity *in vitro* may be metabolized to inactive metabolites *in vivo*. On the other hand, extracts may only show *in vivo* activity due to the metabolism of inactive compounds into active forms.^[12] Hence, the present study was undertaken with a view to evaluate the efficacy of guava leaf extract in the treatment of diarrhea using the *C. rodentium* mouse model.

MATERIALS AND METHODS

Plant material

Collection

Guava leaves (var-Sardar) were collected from Shirwal, Maharashtra, India. The plant material was authenticated by Dr. P. Tetali (Naoroji Godrej Centre for Plant Research, Lawkim Motors Group Campus, Shindewadi, Shirwal, Satara, Maharashtra).

Extraction

A 50% hydroalcoholic extract was prepared and used for the present study. The powdered extract was stored at 4°C.

Reconstitution of the extract

The extract was weighed and reconstituted freshly in D/W (10 mg/mL).

Dose

The dose was 300 mg/kg/day for three consecutive days.

Bacteria

The *C. rodentium* used for the present study (strain ICC 168) was a kind gift from Dr. Gad Frankel, Imperial College, London. A 24 h old culture of *C. rodentium*, grown in Luria Broth under shaking conditions, was used.

Animals

Healthy outbred Swiss albino mice of either sex weighing 20–25 g, aged 8–10 weeks, were used for the present study. The animals were housed under standard conditions of light/dark at a 12/12 h cycle. They were fed with a commercial pellet diet and water *ad libitum*. All animal experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA),

and the study was approved by the Institutional Animal Ethics Committee (FMR/IAEC/MP/01/2011).

Experimental

The experiment was carried out as described by Newman *et al.*^[13] with minor modifications. Briefly, the mice were kept fasting for 12 h prior to the infection and water *ad libitum*. A total of 14 mice were used for the experiment. All mice were fed orally with 250 µL of *C. rodentium* (5×10^8 bacterial cells/mL), grown overnight, using a gavage needle. Twenty-four hours post feeding of the pathogen, fecal samples were collected to ascertain the uptake of infection. Forty-eight hours post infection, the mice that showed infection were divided into two groups, viz., Control and Test.

The mice in the test group were orally fed with 250 µL of guava leaf extract twice a day for three consecutive days. Fecal samples were obtained periodically and homogenized with PBS (1 g feces/10 mL). The samples were serially diluted and plated on Mac Conkey's agar containing ampicillin (180 µg/mL) as a selective medium. The plates were incubated at 37°C for 24 h. The colonies on the plates were enumerated to determine the bacterial load in the fecal sample.

RESULTS

As observed from Figure 1, the feeding of guava leaf extract causes a flush out of *C. rodentium* into the feces on Day 4 in the treated group. The decrease in infection in the treated group was observed from Day 8. Six of seven (85.71%) mice in the test group showed clearance of infection by Day 19. The single mouse in the test group showed clearance of infection by Day 24. In the

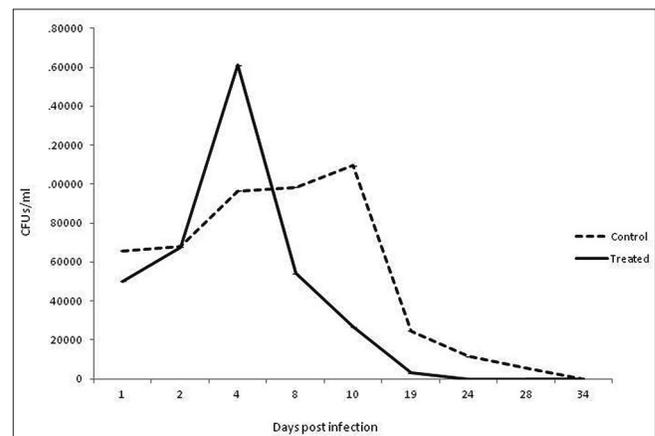


Figure 1: Antidiarrheal efficacy of the *P. guajava* leaf extract in a mouse model. The mice in the test group were treated with the *P. guajava* leaf extract. The clearance of infection in the test group was quicker due to the flush of bacteria post-feeding of the extract. The mice in the control group continued to show infection till Day 29

control group, the decline was observed only post the 10th day of infection, with none of the mice showing clearance of infection by Day 19. The mice in the control group continued to show infection till Day 29. While no statistical test was applied, the delay of 10 days in clearance between the control and the test groups was substantial.

DISCUSSION

The present study is unique in evaluating the role of guava leaf extract for the treatment of infectious diarrhea using a *C. rodentium* model. *C. rodentium* colonizes the lumen of the mouse gut mucosa by formation of A/E lesions on the apical surfaces of the enterocytes,^[14] a feature classically associated with the pathogenesis of EPEC and EHEC. In this respect, *C. rodentium*, EPEC and EHEC share a common virulence strategy. This could thus provide a useful model to evaluate the pathogenesis of acute diarrheal illness and infectious gastroenteritis and for pre-clinical screening of therapeutics.

Adhesion of the organism to the host surface is a crucial early step in colonization of the human gastrointestinal tract by bacteria in all diarrheal infections caused by pathogenic *E. coli*.^[15] The efficacy of guava leaf decoction in inhibiting the bacterial colonization to HEp-2 cells has been reported.^[9] Lectins in guava were shown to bind to *E. coli*, preventing its adhesion to the intestinal wall and thus preventing infection.^[16] The rise in the bacterial load in the fecal sample of the mice in the test group could thus be attributed to the non-adherence of the bacteria to the intestinal wall. We hypothesize that because guava leaves affect the adherence of bacteria, most of *C. rodentium* could have been rendered non-adherent, resulting in the bacteria being flushed out and hence the clearance in the mice in the test group was quicker as compared with those in the control group.

CONCLUSION

While the antidiarrheal activity of guava leaves in animal models used to study physiological diarrhea has been demonstrated earlier, its activity against infectious diarrhea has not been established. The results obtained from the present study demonstrate the antidiarrheal activity of the guava leaf extract using a *C. rodentium* mouse model for infectious diarrhea.

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