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**Pharmacological basis for the medicinal use of *Psidium guajava* leave in hyperactive gut disorders**

## Pharmacological basis for the medicinal use of *Psidium guajava* leave in hyperactive gut disorders

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### Abstract

*Psidium guajava* L. is reputed for its medicinal use in hyperactive gut disorders. This study was aimed to investigate mechanism responsible for its medicinal use in diarrhea and gut spasm. In castor oil-induced diarrheal model, the crude extract of *P. guajava* (100-1,000 mg/kg), provided 20.5-81.1% protection, similar to loperamide. In isolated rabbit jejunum preparations, crude extract was found more potent against high K<sup>+</sup> than spontaneous pre-contractions, similar to verapamil, with EC<sub>50</sub> values of 0.7 (0.4-1.0; n = 8) and 2.3 mg/mL (1.4-3.6; n = 8), respectively, suggests calcium channel blocking activity, as a possible mode of action. The Ca<sup>++</sup> channel blocking activity was further confirmed when pre-treatment of tissue with crude extract (0.3-1 mg/mL) caused a rightward shift in the Ca<sup>++</sup> concentration-response curves, similar to verapamil. Loperamide also inhibited spontaneous and high K<sup>+</sup>-induced contractions and shifted the Ca<sup>++</sup> curves to the right. These data indicate that crude extract of *P. guajava* possesses Ca<sup>++</sup> antagonist-like constituent(s), which explain its inhibitory effect on gut motility.

### Introduction

*Psidium guajava* L. (family; Myrtaceae) commonly known as "guava" is a native of tropical America and has long been naturalized in Southeast Asia. Different parts of the plant have been used in the indigenous system of medicine for the treatment of various human disorders. The decoction of young leaves and shoot is prescribed as spasmolytic in diarrhea (Lozoya et al., 1994; Duke, 2002; de Wet et al., 2010).

Phytochemical analysis has resulted the isolation and identification of various terpenoids, flavonoids and tannins (Lozoya et al., 1994). Two of pentacyclic triterpenoids of the ursane series, namely ursolic acid, 2 $\alpha$ -hydroxyursolic acid, oleanolic acid, maslinic acid and arjunolic acid (Osman et al., 1974) were reported

earlier. Among the major constituents are quercetin, myricetin, luteolin and kaempferol (Gutiérrez et al., 2008).

*Psidium guajava* has been studied for its effect in hyperactive gut disorders, particularly diarrhea and spasm. It has been reported that its leaf extract possesses antidiarrheal (Ojewole et al., 2008) and spasmolytic (Tona et al., 2000) activities. Additionally, the plant extract also possessed, antimalarial (Ponce et al., 1994) activity. In either study, the precise mode of action related to its antidiarrheal and/or antispasmodic effect is not yet clearly understood. This investigation was aimed to explore the underlying mechanism responsible for the medicinal use of *P. guajava* in diarrhea and gut spasm.



## Materials and Methods

### Plant materials and extraction of crude extract

Fresh leaves (20.0 kg) of *P. guajava* were collected from Karachi region and authenticated by Dr. Mohammad Qaiser, Department of Botany, University of Karachi, Karachi, Pakistan. A voucher specimen (KUH-GH No. 53976) has been deposited in the Herbarium of the same Department.

Plant material free of adulterants were repeatedly extracted (3 times) with EtOH at room temperature and the combined extract was evaporated in rotary evaporator at 35-40°C to a semisolid mass, the crude extract of *P. guajava*. The extract was solubilized in normal saline and distilled water for the *in vivo* and *in vitro* experiments, respectively.

### Drugs and standards

The following reference chemicals were obtained from the sources specified: acetylcholine chloride, loperamide hydrochloride, verapamil hydrochloride, potassium chloride (Sigma Chemical Company, U.S.A.) and castor oil (Karachi Chemical Industries, Pakistan). All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh in normal saline on the day of experiment.

### Animals

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Research Council, 1996) and were approved by the Ethical Committee of the Aga Khan University, Karachi, Pakistan. Balb<sup>c</sup> mice (20-25 g) and local rabbits (1.5-2 kg) of either sex used in the study were bred and housed in the animal house of the Aga Khan University under controlled environment (23-25°C). Animals were given tap water *ad libitum* and a standard diet.

### Castor oil-induced diarrhea

The *in vivo* anti-diarrheal activity of the extract was conducted following the methods previously described (Awouters et al., 1978; Jebunnessa, 2009; Shah et al., 2011). In the present study Balb<sup>c</sup> albino mice were fasted for 18 hours. The animals divided in five groups, housed in five steel cages with five in each and the bottom of each cage was covered with blotting sheet. The first group received saline as the vehicle control (10 mL/kg, orally) and acted as the negative control. The doses of the crude extract were selected on a trial basis and administered orally (100, 300 and 1,000 mg/kg) by intra-gastric feeding needle as a suspension to three groups of animals. The fifth group received loperamide (10 mg/kg) orally, which served as a positive control. One hour after treatment, each animal received 10 mL/kg of castor oil orally and was then observed for

defecation. The presence of diarrheal droppings was noted in blotting sheets in the individual cages for 4 hours after challenged with castor oil. Percent protection against the castor oil-induced diarrhea was calculated based on the number of dry feces in each cage in comparison to the wet.

### Isolated tissue preparations

The isolated tissue experiments were carried out, as previously described (Gilani et al., 2005; Shah et al., 2011). Rabbits had free access to water but were fasted for 24 hours before the experiment. The animals were sacrificed by cervical dislocation, the abdomen was cut open and the jejunal portion isolated out. Preparations 2 cm long were mounted in 10 mL tissue baths containing Tyrode's solution maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen (carbogen). The composition of Tyrode's, in mM, was: KCl 2.7, NaCl 136.9, MgCl<sub>2</sub> 1.1, NaHCO<sub>3</sub> 11.9, NaH<sub>2</sub>PO<sub>4</sub> 0.4, glucose 5.6 and CaCl<sub>2</sub> 1.8 (pH 7.4). A preload of 1 g was applied to each tissue and were kept undisturbed for an equilibrium period of 30 min. After equilibration, control responses to a sub-maximal concentration of acetylcholine (0.3 mM) were obtained and the tissue presumed stable only after the reproducibility of the said responses.

Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing the relaxant (spasmolytic) activity directly without the use of an agonist or spasmogen (Gilani et al., 2005).

### Determination of calcium antagonist activity

To assess whether the spasmolytic activity of the test substances was mediated through Ca<sup>++</sup> channel blockade, high concentration of K<sup>+</sup> (80 mM), as KCl, was used to depolarize the preparations (Farre et al., 1991), which produced a sustained contraction. Plant extract and standards were then added in a cumulative fashion to obtain concentration-dependent inhibitory responses. The relaxation of intestinal preparations, pre-contracted with K<sup>+</sup> was expressed as percent of the control pre-contraction.

To confirm the Ca<sup>++</sup> antagonist activity of test substances, the tissues were allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca<sup>++</sup>-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove Ca<sup>++</sup> from the tissues. This solution was further replaced with K<sup>+</sup>-rich and Ca<sup>++</sup>-free Tyrode's solution, having the following composition: KCl 50, NaCl 91.04, MgCl<sub>2</sub> 1.05, NaHCO<sub>3</sub> 11.90, NaH<sub>2</sub>PO<sub>4</sub> 0.42, glucose 5.55 and EDTA 0.1 mM. Following an incubation period of 30 min, control concentration-response curves of Ca<sup>++</sup> were obtained. When the control curves of Ca<sup>++</sup> were found superimposable (usually after two cycles), the tissue were then pretreated with the plant extract for 60 min to test

the possible Ca<sup>++</sup> channel blocking effect. The Ca<sup>++</sup> curves were reconstructed in the presence of different concentrations of the test material.

### Statistics

All the data expressed are mean  $\pm$  standard error of the mean (SEM) and the median effective concentrations (EC<sub>50</sub>) values are given with 95% confidence intervals (CI). The statistical parameter applied is the student t-test with  $p < 0.05$  noted as significantly different (GraphPad program, GraphPad, San Diego, Ca, USA) and concentration response curves were analyzed by non-linear regression (GraphPAD program).

## Results and Discussion

Based on the medicinal use of *P. guajava* in hyperactive gut disorders, such as diarrhea and spasm (Lozoya et al., 1994; Duke, 2002; de Wet et al., 2010), its crude extract was screened for antidiarrheal effect in mice. The crude extract provided protection from diarrhea in castor oil-induced diarrhea, similar to loperamide, a standard antidiarrheal agent (Reynolds et al., 1984). Both extract and loperamide inhibited significantly ( $p < 0.05$ ) the frequency of defecation as well as wetting of feces when compared with the untreated group (i.e. mice received neither crude extract, nor loperamide, but castor oil only). The percent protection provided by the crude extract and loperamide was 20.5-81.1% and 97.3%, respectively (Table I). Verapamil, a standard calcium channel blocker (Godfraind et al., 1986) also provided protection (35.6-82.3%) from castor oil-induced diarrhea.

The induction of diarrhea by castor oil results from the action of ricinoleic acid formed in the hydrolysis of the oil (Iwao and Terada, 1962), which produces changes in the transport of water and electrolytes resulting in a

hypersecretory response and generation of a giant contraction of the intestine (Crocì et al., 1997). Thus, a potential antidiarrheal agent may exhibit its antidiarrheal effect by inhibiting either gut motility and or electrolyte out flux (diarrheal droppings; Crocì et al., 1997). The protective effect of the crude extract against the castor oil-induced diarrhea in mice, similar to loperamide and verapamil, suggests that it has an inhibitory effect either on contraction and or on electrolyte out flux.

There are studies available on the antidiarrheal and antispasmodic activities of the crude extract of *P. guajava* showing that the antidiarrheal effect of the extract was attributed either to its antibacterial (Coutino et al., 2001; Neira and Ramirez, 2005), antisecretory (Gutierrez et al., 2008) or spasmolytic (Morales et al., 1994; Ojewol et al., 2008) activities. Morales et al. (1994) reported that the spasmolytic effect of quercetin, one of the major constituents of all parts of *P. guajava*, may be responsible for the intestinal inhibitory activity and hence for the antidiarrheal action. However, Ojewol et al. (2008) attributed atropine-like spasmolytic activity of crude extract to its antidiarrheal effect. To probe the precise mechanism of the spasmolytic effect of the extract, further study was carried out on gut motility in isolated spontaneously contracting rabbit jejunum, where cumulative addition of crude extract, loperamide and verapamil caused concentration-dependent inhibition of the spontaneous contractions (Figure 1), with EC<sub>50</sub> values of 2.28 mg/mL (1.4-3.6), 1.6  $\mu$ M (1.2-2.2) and 0.2  $\mu$ M (0.1-0.3), respectively (Figure 2). This shows smooth muscle relaxant (spasmolytic) activity. The contraction of smooth muscle preparations, including rabbit jejunum, is depend upon an increase in the cytoplasmic free [Ca<sup>++</sup>], which activates the contractile elements (Karaki and Weiss, 1983). The increase in intracellular Ca<sup>++</sup> occurs either via influx through voltage-dependent Ca<sup>++</sup> channels or its release from

Table I

Effect of the crude extract of the leaves of *Psidium guajava*, verapamil and loperamide on castor oil-induced diarrhea in mice

Group	Dose	Total number of feces in 4 hours	Total number of wet feces in 4 hours	Protection (%)
Control (saline)	10 mL/kg	14.8 $\pm$ 1.1	0.2 $\pm$ 0.3	99.3 $\pm$ 0.9
Castor oil	10 mL/kg	11.8 $\pm$ 0.8	11.6 $\pm$ 0.8	1.3 $\pm$ 0.8
+ extract	100 mg/kg	19.8 $\pm$ 1.0	14.2 $\pm$ 1.0	20.5 $\pm$ 1.2 <sup>a</sup>
+ extract	300 mg/kg	20.8 $\pm$ 1.1	9.2 $\pm$ 0.6	39.7 $\pm$ 3.2 <sup>b</sup>
+ extract	1,000 mg/kg	13.8 $\pm$ 1.2	2.4 $\pm$ 0.9	81.1 $\pm$ 3.2 <sup>c</sup>
+ verapamil	3 mg/kg	11.8 $\pm$ 1.7	64.5 $\pm$ 0.9	35.6 $\pm$ 3.5 <sup>a</sup>
+ verapamil	10 mg/kg	13.8 $\pm$ 1.7	40.4 $\pm$ 4.6	59.6 $\pm$ 4.6 <sup>b</sup>
+ verapamil	30 mg/kg	18.2 $\pm$ 3.3	17.7 $\pm$ 4.5	82.3 $\pm$ 4.5 <sup>c</sup>
+ loperamide	10 mg/kg	8.8 $\pm$ 1.2	0.4 $\pm$ 0.3	97.3 $\pm$ 1.7

Mean  $\pm$  SEM; n = 5; <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.001$  vs. control, Student's t-test

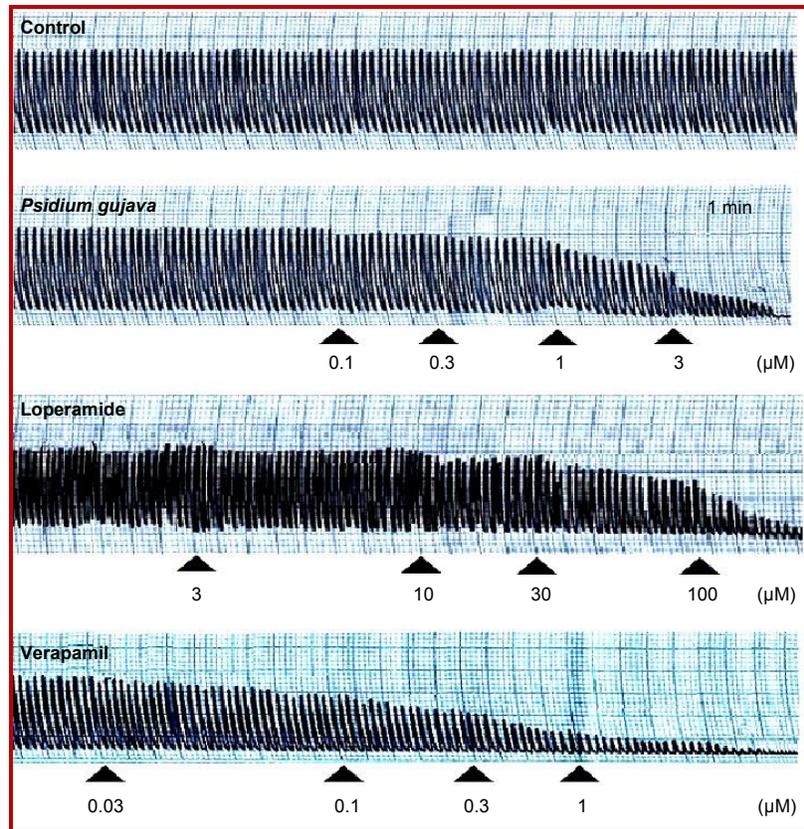


Figure 1: A typical tracing showing the spasmolytic effects of the crude extract of *P. guajava*, loperamide and verapamil on spontaneous contractions in isolated rabbit jejunum preparation

intracellular stores in the sarcoplasmic reticulum. Periodic depolarization and repolarization regulates the spontaneous movements of the intestine and at the height of depolarization, the action potential appears as a rapid influx of  $\text{Ca}^{++}$  via voltage dependent channels (Brading, 1981). Thus, the inhibitory effect of crude extract on spontaneous movements of rabbit jejunum may appear to be due to a CCB effect, possibly, due to interference of  $\text{Ca}^{++}$  influx through  $\text{Ca}^{++}$  channels.

We previously observed that the spasmolytic constituents present in different medicinal plants mediate their effect usually through a CCB effect (Gilani et al., 2005; Shah et al., 2010; Shah et al., 2011). To see, whether the spasmolytic effect of the *P. guajava* observed in this study is also mediated through a  $\text{Ca}^{++}$  antagonist-like effect, a high concentration of  $\text{K}^+$  (80 mM) was added to the tissues bath, which is known to produce sustained contraction through opening of voltage dependent  $\text{Ca}^{++}$  channels. The crude extract was then added in a cumulative fashion, where it was found more potent against high  $\text{K}^+$  pre-contractions than spontaneous contractions, with respective  $\text{EC}_{50}$  value of 0.7 mg/mL (0.4-1.0), similar to verapamil and loperamide (Figure 2). A substance, which can inhibit high  $\text{K}^+$ -induced contractions is therefore, possibly considered to be a  $\text{Ca}^{++}$  antagonist (Godfraind et al., 1986). Thus, the inhibition of high  $\text{K}^+$

pre-contractions of rabbit jejunum by crude extract may reflect the restricted  $\text{Ca}^{++}$  entry via voltage-dependent channels. This hypothesis was further strengthened when pre-treatment of the tissues with plant extract (0.3 -1 mg/mL) caused a rightward shift in the  $\text{Ca}^{++}$  curves (Figure 2), similar to verapamil. Pretreatment of the tissues with loperamide also caused a rightward shift in the  $\text{Ca}^{++}$  curves (Figure 2), which is in accordance to its known  $\text{Ca}^{++}$  antagonist effect at antidiarrheal doses (Reynolds et al., 1984). These data indicate that plant extract possesses a  $\text{Ca}^{++}$  antagonist effect, similar to verapamil or loperamide and thus provides sound pharmacological basis to its antidiarrheal and antispasmodic effects, as the  $\text{Ca}^{++}$  antagonists are considered useful in diarrhea and gut spasms (Brunton, 1996). This is the first functional study of its kind on the gut motility with possible mode of action explored and the earlier studies on antibacterial action of the plant is of added benefit to the plant, thus making the plant more effective in a diarrhea of wide range etiology including infectious and non-infectious.

In summary, this study shows that the crude extract of *Psidium guajava* possesses antidiarrheal along with antispasmodic effects, mediated possibly through  $\text{Ca}^{++}$  channel blockade, which provides sound pharmacological base to its medicinal use in diarrhea and gut

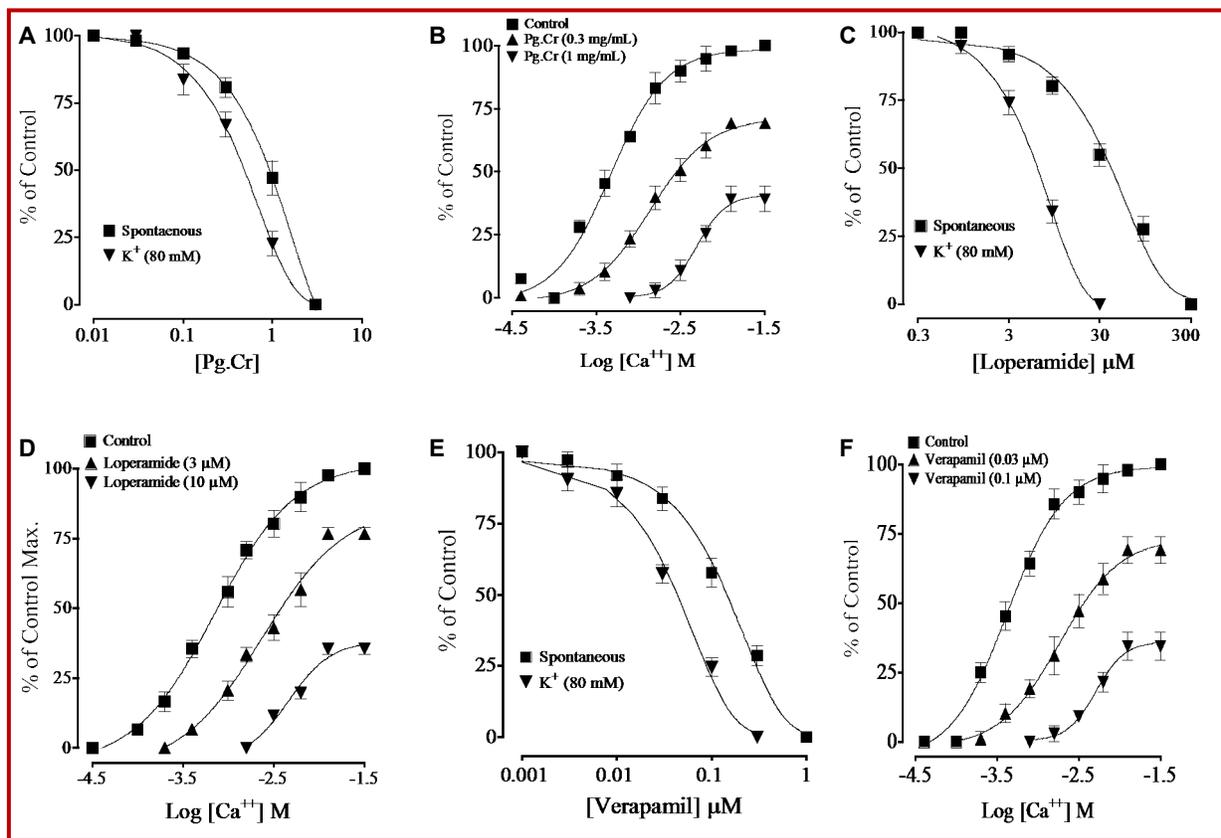


Figure 2. Concentration-response curves of; (A) the crude extract of *Psidium guajava* (Pg.Cr), (C) loperamide, and (E) verapamil on spontaneous and high K<sup>+</sup> (80 mM)-induced contractions. (B, D and F) depict the effect on Ca<sup>2+</sup> concentration-response curves in the absence and presence of different concentrations of Pg.Cr, loperamide and verapamil, respectively, in isolated rabbit jejunum preparations. Values shown are  $\pm$  SEM (n = 5-7)

spasms, though additional mechanism(s) cannot be ruled out.

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