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Antidiabetic and antidiarrhoeal effects on ethanolic extract of *Psidium guajava* (L.) Bat. leaves in Wister rats

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PEER REVIEW

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Comments
This is a valuable research work in which the authors have demonstrated the antidiabetic and antidiarrhoeal activity in guava. The experimentation, *in vivo*, is correctly employed and with appropriate controls.
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ABSTRACT

Objective: To evaluate the antidiabetic and the antidiarrhoeal effects of ethanolic extracts of *Psidium guajava* leave (EEPGL) in Wister rats to support its traditional uses.
Methods: Oral glucose tolerance test model and alloxan induced diabetic test model were performed to evaluate antidiabetic activity of EEPGL at doses of 1.00, 0.50 and 0.75 g/kg respectively. For antidiarrhoeal effects of EEPGL, castor oil-induced diarrhoea model and gastrointestinal motility test with barium sulphate milk model were also assessed at doses of 750, 500 and 250 mg/kg, respectively.
Results: Administration of EEPGL at doses 1.00 and 0.50 g/kg significantly (P<0.05) decreased blood glucose levels in oral glucose tolerance test model as well as 0.75 g/kg dose in alloxan induced diabetic test model in Wister rats (P<0.001). Application of EEPGL at doses of 750 and 500 mg/kg showed antidiarrhoeal effect in castor oil-induced diarrhoeal model (P<0.001 and P<0.01, respectively), and 750 mg/kg (P<0.01), 500 and 250 mg/kg (P<0.05) doses in barium sulphate milk model in aforesaid animals.
Conclusions: These results exhibited the significant antidiabetic and antidiarrhoeal activities of ethanolic extracts of *Psidium guajava* leave in Wister rats.

KEYWORDS
Psidium guajava, Leave extract, Antidiabetic, Antidiarrhoea, Wister rats

1. Introduction

Diabetes is a major disease about 10% of the total population which is caused by a metabolic disorder characterized by fast elevation of blood sugar level. Due to introduction of hypoglycemic agents, diabetes and the related complications continue to be a major medical problem[1]. It is estimated 171 million people suffering from diabetes in 2000 and possibility to increase this value up to 366 million in 2030. In addition, diarrhoeal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year[2,3].

It is reported that- approximately 80 percent of the populations in some Asian and African countries presently use herbal medicine for some aspect of primary health care. It also encouraged studies for the treatment and prevention of diabetes and diarrhoeal diseases depending on traditional medical practices[4,5].

Guava [*Psidium guajava* (L.) Bat (*P. guajava*)] belonging to family Myrtaceae is a traditionally used plant because of its nutrition value. Guava is widely grown in tropical areas like India, Bangladesh, Florida, and West Indies. Different parts of guava are

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reported to be used in folk medicine. In particular, the leaf extract of guava has traditionally been used for the treatment of diabetes in East Asia and other countries[6-8].

P. guajava is also a relatively well-studied species with respect to diarrhoea[9,10]. In Bangladesh, tribes and traditional medicine practitioners use local plants for treatment of various diseases[11]. In addition, for millions who live in remote villages amid the constant risk of recurrent cholera and diarrhoea, the boiling of a few tender guava leaves, which are available in every household, together with rice powder, can be a life-saving solution for preventing cholera related death[2,12]. A few workers reported on guava plants under consideration of its medicinal and economic importance[13,14]. The aim of the present study is to evaluate of pharmacological effects on ethanolic guava leaf extract.

2. Materials and methods

2.1. Plant material and extraction

P. guajava leaves were collected from the Chittagong University Campus, Chittagong, Bangladesh in August 2012. The plant was taxonomically identified by Professor Dr. Mostafa Kamal Pasha, Department of Botany, University of Chittagong and the voucher specimen (Pharma-0024/2012) was deposited at Pharmacological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Chittagong, Bangladesh. The collected leaves were washed, chopped, dried, powdered and extracted with 98% ethanol and concentrated by using rotary vacuum evaporator. The yield of the extract was 3.52% (w/w, in terms of dried starting material) which was kept in refrigerator at 4 °C.

2.2. Experimental animals

Male Wister rats weighting (180±10) g were procured from animal house of BCSIR Laboratories Chittagong to assess the antidiabetic and the antidiarrhoeal activity. All animals were kept under standard laboratory conditions. The animals were fed with standard diet and allowed to drink water *ad libitum*. All aspects of animal care complied with the ethical guidelines of Pharmacology Research Division, BCSIR Laboratories Chittagong, Bangladesh.

2.3. Chemicals

All chemicals and drugs of this experiments such as castor-oil (Shengyang Kaiyingsheng Chemical Co. Ltd., China), Tween 80 [Polyoxyethylene (20), Loba Chemie Pvt. Ltd., India], alloxan tetrahydrate (Merck, India), glibenclamide (Marion Roussel Ltd., Aventis, Bangladesh), loperamide (Opsonin, Bangladesh), glucose powder (dextrose monohydrate, GlaxoSmithKline, Chittagong, Bangladesh Ltd.), barium sulfate ($BaSO_4$) (Merck, India Ltd.) and diethyl ether (Sigma-Aldrich, India) were obtained commercially and were of analytical grade.

2.4. Experiments

2.4.1. Oral glucose tolerance test (OGTT) model

The OGTT was performed in overnight fasted (18 h) normal rats as per reported method[15]. Firstly, blood glucose level (BGL) of all fasted rats was withdrawn from the tip of tail and measured as "0 min" with the help of a blood glucose meter (Accu-Chek Active, Roche Diagnostics, Germany). Then, they were divided into four groups (n=5). Control animals received only distilled water (2 mL per rat) as Group I. Group II rats were given glibenclamide orally at a dose level 4.15 mg/kg body weight as positive control. Wister rats in Group III and IV were treated orally with EEPGL at doses of 1.0 and 0.5 g/kg body weight, respectively. After 30 min, BGL of all groups was measured. Then these animals were given glucose solution (10 g/kg body weight) orally and the concentration of BGL was estimated at 30, 60, 120 min subsequently.

2.4.2. Alloxan induced diabetic test model

After fasts of 18 h, forty two male Wister rats were induced by alloxan tetrahydrate (100 mg/kg *i.p.*) in sterile saline. Diabetes was confirmed after 24 h with BGL of >10 mmol/L. Then the diabetic animals were separated and used for the study. Fifteen diabetic rats were selected and randomly divided into three experimental groups (marked as Group II to IV). Each group contains five rats. Group II as diabetic control received only distilled water. Group III as positive control was treated antidiabetic drug glibenclamide (4.15 mg/kg). Group IV was treated with ethanolic extracts of *P. guajava* leave (EEPGL) at 750 mg/kg. Group I was previously selected as control group which was non diabetic. After extract/drug administration, BGL of samples was determined at 0 h, 3 h, 6 h and 9 h by aforesaid system. All the animals were anesthetized with diethyl ether during blood collection. Room temperature and humidity were maintained in order to increase the survival capacity of treated rats.

2.4.3. Castor oil-induced diarrhoea (COID) model

To determine antidiarrhoeal activity of EEPGL, COID model was conducted by following described method[16]. Twenty five Wister rats were randomly divided into five equal groups (n=5) namely control group, positive control group and three treated groups. The control group received only distilled water 2 mL per rat while positive control group received loperamide 2 mg/kg as standard and three treated groups received EEPGL at the dose of 750 mg/kg, 500 mg/kg and 250 mg/kg body weight, respectively. Rats were housed in separate cages with paper placed below for collection of fecal matters. Firstly, extract and drug were given orally to treated groups and positive control group respectively. In control group, only distill water was given orally. Then, one hour later castor oil (2 mL per rat) was induced orally to all rats initiating diarrhoea. The number of both hard and soft pellets was counted at every hour

over 6 h period for each rat. Diarrhoea was defined as the presence of stool with fluid material that stained the paper placed beneath the cages.

2.4.4. Gastrointestinal motility test with $BaSO_4$ milk (BSM) model for diarrhoea

BSM model was carried out by reported method[17]. Overnight fasted (18 h) twenty five Wister rats were randomly divided in to five equal groups (n=5). Control group received only distilled water 2 mL per rat orally. Positive control group received commercially available anti diarrhoeal drug loperamide 2 mg/kg orally. Treated groups received EEPGL 250 mg/kg, 500 mg/kg and 750 mg/kg orally. After thirty minutes, all groups of rats were administered with 2 mL of 10% $BaSO_4$ solution. Lastly, after 30 min rats were sacrificed. Finally, the distance traveled by $BaSO_4$ milk was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileo-cecal junction).

2.5. Statistical analysis

All the values of antidiarrhoeal, tests were expressed as mean± SEM (standard error of the mean). Statistical differences between the mean of the various groups were analyzed by using student's t test. All the graphical presentation and statistical calculations were prepared using "Microsoft Excel-2007". Mean values were considered significantly different if P<0.05, P<0.01 and P<0.001.

3. Results

3.1. Effect of EEPGL on OGTT model

The results of OGTT of positive control and different doses of EEPGL on Wister rats are shown in Table 1. All the doses of EEPGL and positive control were effective to the decreased BGL with increasing exposure time. Positive control exhibited the lowest BGL in all intervals, whereas among the different doses of EEPGL 1 g/kg shown lower BGL at all intervals. The BGL in the positive control were 4.54, 3.60, 8.66, 6.42 and 2.88 mmol/L at different administrations shown in Table 1. From results significant (P<0.05) BGL reductions were at 1 g/kg and 0.5 g/kg doses of EEPGL.

Table 1
Effect of EEPGL on oral glucose tolerance test.

Groups	Blood glucose concentration (mmol/L)				
	Fasting	Treatment[a]	Glucose administration		
	0 min	30 min	30 min	60 min	120 min
Group I	4.18±0.34	4.56±0.16	12.70±1.21	12.08±1.13	10.10±0.58
Group II	4.54±0.45	3.60±0.34*	8.66±0.58*	6.42±0.58**	2.88±0.34***
Group III	4.04±0.25	4.50±0.31	11.52±2.55	7.62±1.21*	4.86±1.12*
Group IV	4.50±0.30	5.10±0.27	11.88±2.40	9.50±2.33	7.10±0.90*

Values are expresed as mean±SEM. a: Group I was treated with stilled water, Group II with glibenclamide, Group III and IV with EEPGL at doses of 1.0 and 0.5 g/kg body weight, respectively. *: P<0.05, **: P<0.01, ***: P<0.001 means significant difference compared control with positive control and extract.

3.2. Effect of EEPGL on alloxan induced diabetic test model

The mean blood glucose concentration of control, diabetic control, positive control and plant extract on alloxan induced diabetic rats were estimated at 0, 3, 6 and 9 h, respectively as shown in Table 2. After the administration of alloxan, BGL increased. The mean BGL in the case of alloxan induced diabetic rats were 19.46, 22.72, 24.90 and 29.03 mmol/L at the 0, 3, 6 and 9 h, respectively. After 3 h of the administration in Groups III and IV the BGL of treated rats gradually decreased shown in Table 2. Extracts of *P. guajava* and positive control had equally effective in reduction of BGL in alloxane induced diabetic rats (P<0.001 and P<0.01).

Table 2
Effect of EEPGL on alloxan induced model.

Test samples	Mean of blood glucose concentration (mmol/L)			
	0 h	3 h	6 h	9 h
Group I	6.78±0.29	6.54±0.51	6.70±0.38	6.14±0.22
Group II	19.46±0.59$^{###}$	22.72±0.99$^{###}$	24.90±1.06$^{###}$	29.03±1.81$^{###}$
Group III	21.44±1.52	16.14±1.74	9.30±1.81**	5.36±0.59***
Group IV	21.52±0.63	18.80±0.82**	10.74±0.45***	7.22±0.53***

Values are expresed as mean±SEM; $^{###}$: P<0.001 means significant difference compared control with diabetic control. *: P<0.05, **: P<0.01 and ***: P<0.001 refer to diabetic control compared with positive control and extract.

3.3. Effect of EEPGL on COID model

The results about antidiarrhoeal effect of loperamide and EEPGL in COID on Wister albino rats are shown in Table 3. The results indicated that both *P. guajava* leaf extracts and loperamide inhibited significantly the frequency of defecation and wetted feces when compared to the control group and treated group (P<0.001 and P<0.01).

Table 3
Effect of EEPGL on COID model.

Group		Total faeces in 6 h	% Inhibition of defecation	No. of wet faeces in 6 h	% Inhibition of defecation
Control		21.40±2.07		19.80±3.63	
Positive control (loperamide)		5.20±0.84***	76	2.60±0.89***	87
Leaves extract	750 mg/kg	10.20±1.48***	52	7.40±1.14***	63
	500 mg/kg	15.80±2.28**	26	11.20±1.48**	43
	250 mg/kg	19.20±4.87	10	16.60±3.65	16

Values are expresed as mean±SEM; **: P<0.01 and ***: P<0.001 significant compared control with positive control and extract.

3.4. Effect of EEPGL on BSM model

The results about gastrointestinal motility test with BSM of EEPGL and loperamide on Wister rats are shown Table 4. Both treated groups of *P. guajava* leaf extracts and loperamide significantly decreased the gastrointestinal motility of rats in BSM model at 30 min study. In addition, percentages of inhibition in three treated groups compared to control group are 37.83%, 30.01% and 29.56% at doses of 750, 500 and 250 mg/kg, respectively, while positive control exhibits 39.60% inhibition.

Table 4
Effect of EEPGL on gastrointestinal motility with BSM on rats.

Group		Length of gastrointestinal tract (cm)	Distance passed by $BaSO_4$ cm (Mean±SEM)	$BaSO_4$ transverse (%)	Inhibition (%)
Control		117.80±6.80	69.00±5.00	58.57	
Positive control (loperamide)		113.70±4.50	40.20±12.10	35.38***	39.60
P. guajava L.	750 mg/kg	115.20±7.02	41.60±13.59	36.41**	37.83
	500 mg/kg	113.00±7.60	46.20±22.65	40.99*	30.01
	250 mg/kg	111.00±12.47	45.80±9.02	41.26*	29.56

Values are expresed as mean±SEM; *: $P<0.05$, **: $P<0.01$ and ***: $P<0.001$ refer to significant difference compared control with positive control and extract respectively.

4. Discussion

The leaf extract of *P. guajava* stimulated glucose metabolic enzymes in liver tissues[6,19]. However, treatment with freshly prepared EEPGL significantly reduced BGL and lipid profile levels in diabetic albino rats and had similar effect in diabetic patients[20]. The results in this study revealed the antidiabetic activity of selected EEPGL which is significant at positive control ($P<0.01$ and $P<0.001$). The present study also supports most of the previous reports shown that guava leaf can decrease BGL[19,21-24]. Though the antidiabetic activity of EEPGL was diverse at different treated doses in the present study, it supported the folklore claim on antidiabetic activity of these plants[8].

P. guajava leaf aqueous extract (50–400 mg/kg, *p.o.*) produced dose dependent and significant ($P<0.05$–0.01) protection of rats and mice against COID[25]. In the same model, the crude extract of *P. guajava* (100-1 000 mg/kg), provided 20.52%-81.05% protection, similar to loperamide[26].

In the present study, the EEPGL was exhibited antidiarrhoeal effects in Waster rats. It was found to be significantly different at $P<0.01$ and $P<0.05$ with respect to control. The crude extract provided protection from diarrhoea in COID, similar to loperamide, a standard antidiarrhoeal agent[27]. Some authors reported positively for *P. guajava* leaf extract in hyperactive gut disorders which has been supported by the present study[28,29]. In addition, it also reported that EEPGL protected diarrhoea up to level of 55.6%[2]. These results agree with similar reports which have established reduction in gastric motility as being the mechanism by which many antidiarrhoeal agents act[30].

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

From ancient times, about 10% of the adult population in the world suffer diabetes. Other important disease diarrhoeal is responsible for death of million of people each year. Plants are used as medicine from ancient times. At the present time studies on the medicinal activity of plants was intensified all over the world. The purpose of this work is to evaluate the pharmacological effects of ethanolic extracts of *P. guajava* leaves.

Research frontiers

In the present research the authors performed the study of antidiabetic and antidiarrhoeal activities of ethanolic extracts of guava leaves, *in vivo*, on Wister rats. They demonstrated significant antidiabetic and antidiarrhoeal activities of the extracts that are the new contribution to the knowledge of guava biological activities.

Related reports

There are many studies on different aspects of *P. guajava* biological activities but this paper is a new contribution to the antidiabetic and antidiarrhoeal activities of the ethanolic leaf extracts of the plant.

Innovations and breakthroughs

The paper demonstrated the possible use of guava as antidiabetic and antidiarrhoeal with *in vivo* experiments. The methodology is modern and clearly applied to demonstrate the purposes of the research.

Applications

As what was mentioned in the paper, the ethanolic leaf estracts can be use for the development of medicines.

Peer review

This is a valuable research work in which the authors have demonstrated the antidiabetic and antidiarrhoeal activity in guava. The experimentation, *in vivo*, is correctly employed and with appropriate controls.

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