RESEARCH ARTICLE

Antidiarrheal Activity of Ethanolic Fruit Extract of Psidium Guajava (Guava) in Castor Oil Induced Diarrhea in Albino Rats

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ABSTRACT

Background: Diarrhea is one of the common health conditions affecting people in less developed countries. The World Health Organization (WHO) has estimated that 1.5 billion episodes of diarrhea occur every year in developing countries, resulting in 3 million deaths.

Aims & Objective: This study was done to evaluate anti-diarrheal potency of the ethanolic fruit extract of Psidium guajava using Wistar albino rats to scientifically validate its continued use by the local people. I was also aimed at determining the acute toxicity in mice and phytochemical composition of the extract.

Materials and Methods: The fruits were collected in July, 2012 from the College of Veterinary Medicine farm, Makerere University, dried for 1 week, ground and macerated in 70% ethanol. The dry extract was reconstituted using normal saline and orally administered to different groups of rats at doses of 200, 400 and 600 mg/Kg. Anti-diarrheal activity was determined using the percentage reduction in the frequency of defecation in rats with castor oil-induced diarrhea. Loperamide (1 mg/Kg) was used as positive control. Phytochemical composition was qualitatively determined as described by Harborne (1998). Acute toxicity was evaluated by determination of LD_{50} and observations of toxic signs.

Results: The ethanolic crude fruit extract of Psidium guajava showed significant ($p<0.05$, Dunnet test) antidiarrhea activity evidenced by the reduction in rate of defecation by up to 78.33% at 600mg/kg body weight comparable to loperamide (100%). This activity could be attributed to the phytochemicals such as flavonoids and tannins in Psidium guajava that were present in high levels and have been reported to exhibit antidiarrheal activity through denaturing protein hence forming protein tannates which minimize the intestinal mucosa permeability. The LD_{50} of the crude ethanolic fruit extract was 10,715 mg/Kg.

Conclusion: The findings of this study show that the fruit of Psidium guajava have a very significant antidiarrhea activity and are safe to use as indicated by the high LD50 value. This supports the traditional use of the ethanolic fruit extract of Psidium guajava as herbal remedy for treatment of diarrhea.

KEY WORDS: Psidium Guajava; Fruit Extract; Phytochemicals; Antidiarrheal Activity

INTRODUCTION

Each year there are approximately 4 billion cases of diarrhea worldwide. According to the World Health Organization (WHO), diarrhea still accounts for 1.6 -2.5 million deaths annually and each child in the developing world experiences an average of three episodes of diarrhea per year.[1] The World Health Organization (WHO) has estimated that 1.5 billion episodes of diarrhea occur every year in developing countries, resulting in 3 million deaths[2] and 5,483 deaths every day[1]. In Africa, diarrhea is four times more common among children with HIV and seven times more common among adults with HIV than their HIV-negative household members.[3] Amongst the poor and especially in the developing countries, diarrhea is a major killer. In 1998, diarrhea was estimated to have killed 2.2 million people, most of whom were under 5 years of age.[1] Clearly, despite the decline in diarrhea mortality, diarrhea remains one of the principal causes of morbidity and mortality in child.[1]

The majority of people living in rural areas almost always use traditional medicines to treat all types of diseases including diarrhea. Many plants available in Uganda, namely Psidium guajava, Pseudathri hookeri, Capiscus frutescens, Cannabis sativa, Hibiscus aponeurus, Toddalia asiatica, Bidens pilosa, Vernonia amygladina, Rhus vulgaris, Hypericum peplidifolium, Paullia pinnata, Canthiium gueinxii, Maesa lanceolata, Leonotis mollissima, Annona senegalnesis, Ehretia cymosa, Lippia javanica, are used in traditional folklore medicine for treatment of diarrhea.[5]

In the past 2 decades, there has been a search for drugs that might inhibit the process of diarrhea development especially the secretory process. Although a number of drugs have emerged, none has found a place in the routine management of diarrhea.[5] Local herbalists have depended on medicinal plants as reliable means of treating diarrhea. Hence the use of medicinal plants that possess anti-diarrheal activities has been explored as a measure that could be of benefit in combating widespread diarrhea infection especially in third world countries.[1]

MATERIALS AND METHODS

Study Design

This was an experimental study in which both qualitative and quantitative data was obtained on the effect of different doses of the ethanolic crude extract of Psidium guajava fruit on the number of wet faeces in castor oil induced diarrhea in Wistar albino rats. Acute toxicity was also evaluated in laboratory mice by determination of LD50 and the phytochemical composition of the extract from the plant was determined. All these activities took place in July of 2012.

Rat Selection

The experiment involved 30 Wistar albino rats of either sex weighing between 100-150g. The rats were obtained from the Division of Pharmacology, Department of Physiological Sciences of the College of Veterinary Medicine, Makerere University. The animals were allowed to acclimatize for 2 weeks prior to the study. They were housed in a clean wire mesh cages (10 rats per cage) and maintained under standard laboratory condition of 12 hours natural light and 12 hours darkness at ambient room temperature. The rats were fed on pellets supplied by Ugachick (U) Ltd, and water was made available ad libitum.

Plant Collection & Pre-Extraction Procedures

Fruits of Psidium guajava were collected from the farm at the Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity- Kampala. The Fruits were chopped into small pieces and dried in the shade for one week. The dried fruits were reduced to powder mechanically using mortar and pestle and stored in a black polythene bag in locked cupboard.

Extraction Process & Storage of the Extract

The powder was extracted through cold maceration by soaking 300g of powdered fruit tissue in 1070 ml of 70% aqueous solution of

ethanol (Sd fine-Chem Ltd, India) in a brown bottle for three days, with occasional shaking each day and then filtered using cotton wool. Weighing was done using analytical weighing balance (TYPE PJ 3000-METTLER, Switzerland) whereas the volume of ethanol was measured with measuring cylinders and beakers. The filtrate was concentrated by using a rotary evaporator (CH-9230 Flawl/Scwel) then the concentrate was kept in oven at 45˚C for 12hr to obtain a semi-solid extract that was stored at 4˚C in the refrigerator till the end of the study.

Extract Reconstitution & Dosage Formulation

The extract was reconstituted by suspending 4g of crude extract in 20 ml of distilled water to obtain a stock solution of 200 mg/ml. The volume of the extract to be administered was calculated as recommended by Woodson et al., (1987)[6] using the formula:

Volume of the extract (ml) = [Weight of the rat (kg) × Dose rate (mg/kg)] / Stock concentration (mg/ml)

Evaluation of Antidiarrhoeal Activity

Thirty (30) Wistar albino rats were fasted for 18 hr prior to the experiment but given water ad-libitum and divided into five groups of 6 rats each. The rats in group 1 (negative control group), received 1ml of distilled water orally. The rats in groups 2, 3 and 4 were treated with the ethanolic fruit extract of Psidium guajava at doses of 200, 400, and 600 mg/Kg body weight by oral route respectively. The rats in group 5 (positive control group) were treated with loperamide at the dose of 1mg/kg body weight orally. After an hour of dosing, all the rats were treated with 1.5 ml of castor oil orally using a gavage tube to induce diarrhea. The rats were then observed for frequency and consistency of fecal material. The numbers of wet fecal droppings were counted within 4 hours after castor oil administration as recommended by Awouters et al.,(1978)[7] and Gnaasekar et al., (2004)[8]).

Acute Toxicity Test of Psidium Guajava Extract

In this experiment, 30 laboratory albino mice were fasted for 8 hours but given water ad-libitum and were divided into five groups of 6 mice each. The mice in group 1 (negative control group) received 0.5ml of normal saline orally while those in groups 2, 3, 4 and 5 were treated with the ethanolic fruit extract of Psidium guajava at the doses of 5000, 10000, 15000 and 20000mg/Kg respectively prepared from a stock of 400 mg/ml of extract. The number of dead mice per group within 24 hours was recorded and percent mortality calculated. The signs of the toxicity were also observed and recorded.

Phytochemical Screening of Psidium Guajava Fruit Extract

The presence of the following phytochemicals were qualitatively determined from the ethanolic fruit extract of Psidium guajava i.e. tannins, alkaloids, steroid glycosides, flavonoids, saponins and anthracenosides using the method described by Harborne, 1998.[9]

- *Tannins:* One (1) ml of the ethanolic extract was diluted with 1 ml of water and then 3 drops of diluted solution of ferric chloride were added. Presence of tannins was confirmed by the formation of brown greenish colour.
- *Alkaloids:* To 1 ml of the extract few drops of aqueous solution of hydrochloric acid was added followed by 0.5 ml of Mayes reagent. Formation of yellowish white precipitate indicated presence of alkaloids.
- *Steroid Glycosides:* To 1 ml of extract an equal volume of cold concentrated (98%) sulphuric acid was added. Formation of intense red colour indicated presence of glycosides.
- *Flavonoids:* Five (5) ml of the extract was evaporated to dryness. The residue was dissolved in 2 ml of methanol (50%) by heating followed by addition of magnesium metal fillings and 5-6 drops of concentrated hydrochloric acid. A red colour indicated presence of flavonoids.
- *Saponins:* Two (2) ml of diluted solution of the extract with water (1:10) was put in a

test tube of 1.6 cm in diameter and shaken for 2-3 minutes. Formation of froth or foam indicated the presence of the saponins.

- *Anthracenosides:* Four (4) ml of the extract was concentrated to 2 ml in boiling water and then 2 ml of 25% ammonia added with thorough shaking. A cherish red colour of the alkaline solution indicated the presence of emodols (aglycones of anthracenosides) in an oxidized form.

Data Analysis

Data was analyzed using SPSS version 17. The experimental results were expressed as the mean ± S.E.M of five determinations.[6] The ANOVA test was used to assess for any statistically significant differences between the treatment and control groups while the Dunnet method was used to test mean differences. P-values less than 0.05 were considered significant. For LD50 determination, the percent mortality at each dose was transformed into probit as described by Miller and Tainter[10], Ghosh[11] and Al-Ali et al.[12], and presented as mortality probit versus log dose plot. Correlation was done between mortality probits and log dose and the correlation coefficient (R2) determined.

RESULTS

Anti-Diarrheal Activity of Psidium guajava

The mean frequency of wet faecal droppings decreased with increase in dose of the ethanolic fruit extract of Psidium guajava, with the mean frequency of defecation being lower in the group that was treated with 600 mg/Kg and higher in the groups that was treated with 400 mg/Kg and 200 mg/Kg respectively as shown in Table 1. The mean frequency of wet faecal droppings for the rats in the loperamide-treated group was 0.0; hence it totally inhibited diarrhea. The negative control group treated with 1.5 ml distilled water had the highest mean frequency of faeces (Table 1), hence there was no inhibition of diarrhea in this group.

The extract exhibited significant anti-diarrheal activity against castor oil induced diarrhea in laboratory albino rats. There was statistically significant reduction ($p<0.05$) in the number of wet faeces by 78.33% at 600 mg/kg when compared to negative control rats. There was no significant difference ($p>0.05$) in percent reduction of wet faeces for the 200 mg/Kg and 400 mg/Kg groups when compared with the negative control group. The extract thus showed dose dependent inhibition of diarrhea as shown in Table 1. The activity of the extract at 600 mg/Kg was comparable to that of loperamide since there was no significant difference (p-value, 0.082) in the mean number of faecal droppings between these two groups.

Acute Toxicity

The most dominant signs of acute toxicity effects were: laboured breathing, irritation, hind limb paralysis, convulsions and ataxia. The severity of the signs of toxicity increased with increase in the dose. There was no observed sign of toxicity in the group of mice that received normal saline. The lowest observed effect level (LOEL) recorded was at a dose rate of 5000mg/Kg .The number of death reported at LOEL was one mice out of six (16.7%) and the corresponding toxic effects included hypoactivity and laboured breathing. At the highest dose level, all mice in the group (100%) died within 3 hours post-administration and signs of toxicity included ataxia, convulsion, laboured breathing and paralysis of the hind limb. The corresponding mortality probit for determination of LD50 were calculated as recommended by Miller and Tainter[10], Ghosh[11] and Al-Ali et al.[12], and are shown in Table 2.

The mortality was dose dependent (R2 = 0.9444) as shown in the probit against log dose plot (figure 1).The dose of the extract that killed all the mice in a group was 20,000mg/Kg. The calculated LD50 of P. guajava was 10715mg/Kg.

Phytochemical Screening

The results of the phytochemical screening showed that the ethanolic fruit extract of Psidium guajava contained high levels of tannins, steroid glycosides and flavonoids. Alkaloids and saponins were moderately present while

anthracenosides were absent. Table 3; shows the phytochemical profile of ethanolic extract of P. guajava.

Table-1: Effect of the Ethanol Extract of P. Guajava Fruits at Different Dose Levels on Castor Oil Induced Diarrhea

Treatment	No. of wet faeces	% of Inhibition	DF	P-value
200 mg/kg extract + Castor oil	4.67 ± 0.0.95	21.67	5	0.387
400 mg/kg extract + Castor oil	3.33 ± 0.0.49	45.00	5	0.38
600 mg/kg extract + Castor oil	1.33 ± 0.61	78.33*	5	0.04
1 mg/kg Loperamide + Castor oil	0.00 ± 0.00	100.00	5	0.01
1.5 ml normal saline + castor oil	6.0 ± 0.82	0.00	5	-

* p <0.05, when compared with the negative control; Data was expressed as mean ± SEM; n = 6; DF: Degree of Freedom

Table-2: Mortality Data for Ethanolic Fruit Extract of P. Guajava

Dose mg/Kg	Log Dose	Dead/ Total	Dead %	Corrected % Dead	Probits
0.5 ml normal saline	-	0	0	0	0
5,000	3.70	1/6	16.7	16.7	3.51
10,000	4.00	2/6	33.3	33.3	4.59
15,000	4.18	4/6	66.7	66.7	5.43
20,000	4.30	6/6	100	95.8	6.73

N=6 mice per group

Table-3: Results of Phytochemical Profile of Psidium Guajava

Phytochemical	Intensity
Tannins	+++
Alkaloid	++
Steroid glycosides	+++
Flavonoids	+++
Saponins	++
Anthracenosides	-

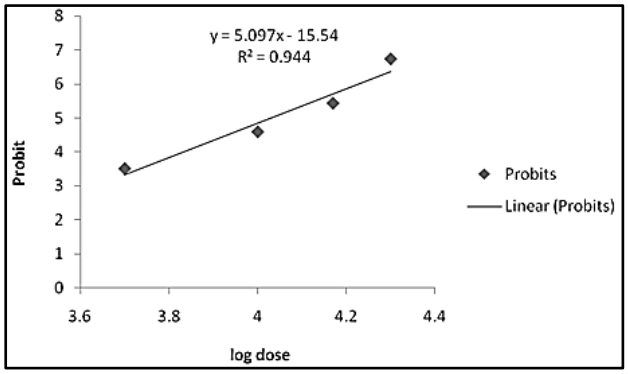

Figure-1: Probit against the Log Dose of P. Guajava (The calculated LD_{50} of P. guajava was 10715 mg/kg)

DISCUSSION

This study involved evaluation of the anti-diarrheal activity of the ethanolic fruit extract of Psidium guajava in castor oil induced diarrhea in albino rats using loperamide as positive control, determined its acute toxicity in mice and its phytochemical composition. The extract exhibited significant anti-diarrheal activity in laboratory albino rats with statistically significant reduction in the number of wet faeces by 78.33% at 600 mg/kg when compared to negative control rats ($p<0.05$). The activity of the extract at 600 mg/Kg was comparable to that of loperamide since there was no significant difference in the mean number of fecal droppings between these two groups ($p>0.05$). The anti-diarrheal activity was dose-dependent.

Phytochemical screening of the extract showed high levels of tannins and flavonoids and these phytochemicals could be responsible for the anti-diarrheal activity observed in this study through inhibition of peristaltic movement. Tannins and tannic acids also denature proteins forming tannates which decrease the intestinal mucosa permeability.[14]

Other studies indicate that flavonoids[15] and alkaloids[16] possess antidiarrhea activity. The antidiarrhea activity of flavonoids has been ascribed to their ability to inhibit peristaltic activity and hydroelectrolyte secretion[17,18], which increase in diarrhea. In-vitro and in-vivo experiments by Sanchez et al.[19], have shown that flavonoids are able to inhibit the intestinal secretory response and induce prostaglandin E2. In addition, flavonoids possess antioxidant properties[20] which are presumed to be responsible for inhibitory effects exerted upon several enzymes including those involved in the arachdonic acid metabolism[21]. Therefore it is possible that the antisecretory, anti-inflammatory and antioxidant properties of flavonoids could be responsible for the antidiarrhea activity of Psidium guajava. Therefore a combination of tannins and flavonoids presumably led to a synergistic anti-diarrheal activity in albino mice.

The LD_{50} of the ethanolic leaf extract of Psidium guajava in mice was determined as 10,715 mg/Kg indicating that the leaves of plant have low toxicity as compared to the value of 5000

mg/Kg which is considered to be practically safe. In addition to the antidiarrheal activity of this plant demonstrated in this study, other studies have shown other medicinal effects such as antiinflammatory, antihemostatic[22,23], antimalarial[24], antimicrobial[25], antifungal[24,26] and anti-diabetic effects[27].

CONCLUSION

The ethanolic fruit extract of Psidium guajava possesses anti-diarrheal activity and this justifies the use of this plant as herbal remedy against diarrhea. The plant can be considered to be safe for use as an anti-diarrhea remedy given the high LD50 of 10715mg/Kg that was determined in this study. The plant contains high levels of phytochemicals especially tannins and flavonoids which could be responsible for its anti-diarrheal activity. Studies need to be done on different fractions of the extract and other part of the plants since in this study only fruits were used. Chromatographic techniques need to be used to obtain pure fractions, as this may lead to an increase in efficacy. Chronic toxicity effect of Psidium guajava needs to be done if it is to be used in management of diarrhea.

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