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Anti-diarrhoeal Activity of *Psidium guajava* (Gauva) Aqueous Leaf Extract in Experimental Animals

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ABSTRACT: Aqueous leaf extract of *Psidium guajava* (Guava) was evaluated for anti-diarrhoeal activity against castor oil-induced *diarrhoea* in albino rats. 50 and 100mg/kg (body weight) doses of the leaf extract showed a dose-dependent anti-diarrhoeal activity comparable to a standard anti-diarrhoeal drug Diphenoxylate given intraperitoneally at 5mg/kg (b.w.) Also, the extract reduced intestinal transit time in charcoal meal test in the same manner. A 100mg/kg dose of the extract also showed an anti-enteropooling effect comparable to a standard anti-enteropooling agent Chlopromazine (20mg/kg, intraperitoneally). Further studies are needed to isolate the effective components of the leaf extract.

Keywords: Anti-diarrhoeal Activity, *Psidium guajava,* Castor oil induced, Diarrhoea

INTRODUCTION

Diarrhoea is a condition in which faeces are discharged from the bowels frequently and in liquid form (Venkatesan *et al.*, 2005) as a result of infectious microorganisms (bacteria, viruses and parasites) and disturbances of the normal functioning of the bowels in absorbing fluids contained within it (Snyder and Merson, 1988).

Diarrhoea has long been recognized as one of the most important health problems in the developing countries (Snyder and Merson, 1988). Worldwide distribution of diarrhoea accounts for more than 5 – 8 million deaths each year in infants and children less than 5 years. According to WHO estimation for the year 1998, there were about 7.1 million deaths due to diarrhoea (Park, 2000).

In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines in treating all sorts of diseases including diarrhoea. It thus becomes important to identify and evaluate commonly available natural drugs as alternative to currently used anti-diarrhoeal drugs, which are not completely free from adverse effects (Hardman and Limbird, 1992).

Psidium guajava (Guava) is a common shade tree or shrub whose fruits are eaten fresh and or made into drinks, ice cream e. t. c. Guava tree is easily identified by its distinctive smooth, copper-coloured bark that flakes off, showing a greenish layer beneath (Smith and Nigel, 1992).

The leaves and bark of the guava tree have a long history of medicinal uses that are still employed today. In fact, an infusion from the leaves and or bark has been used by many tribes for diarrhoea and dysentery throughout the Amazon region, and people also employ it for sore throat, vomiting, stomach upset, for vertigo and to regulate menstrual periods. Tender leaves are chewed for bleeding gums and bad breath and it is said to prevent hangovers (if chewed before drinking). A decoction of the leaves is used topically for wounds, ulcers and skin sores. Guava leaves are still used for diarrhoea in Latin America, Central and West Africa, including Nigeria, where almost all parts of the plant are used in traditional medicine for the treatment of various conditions like cough, dysentery, *diarrhoea* and toothache (Hutchinson and Dalziel, 1964).

The objective of this work was to investigate the possible anti-diarrhoeal, intestinal transit distance and anti-enteropooling effects of the guava leaf extract in albino rats

MATERIALS AND METHODS

Plant material

The aerial parts of *P. guajava* (Guava) were obtained from Runjin Sambo area of Sokoto State. The leaves were dried under shade, each dehydrated leaf powdered to a fine texture, using mortar and pestle, and then sieved. 100g of the powdered extract was dissolved in 250ml

distilled water, in a sterile beaker, heated to boilng for about 30 minutes and allowed to cool (Ajagbonna and Onyeyili, 2002). The boiled extract was then sieved first with muslin cloth and later with sterile cotton wool placed in a sterile glass funnel to separate extract from residues. The filtrate was later oven dried at 60^0C on the day of the extraction. 1g of the dried concentrate was later dissolved in 100ml distilled water in a sterile conical flask and used as a stock solution for the experiment.

Animal

Albino rats of both sexes weighing 130 – 150g were used for the experiments. All animals were fed with standard animal feed (Pfizer) and given tap water *ad libitum* before the experiments. Each experimental group consisted of four animals housed in separate cages.

Castor oil-induced Diarrhoea

Rats for this test were first observed for any wet or coloured droppings on a plain sheet of paper. The rats were grouped in a separate compartment with a white clean paper spread on the floor of the cage to receive the droppings over a period of 24 hours. Those that produced wet droppings were excluded from the experiment.

The rats were then divided into five groups (n = 4) and fasted for 18 hours. The aqueous leaf extract of *P. guajava* (50 and 100mg/kg p. o.) were administered orally to the first two (2) groups of rats. The third group received standard anti-diarrhoeal drug Dipheoxylate (5mg/kg) intraperitoneally, as positive control. The remaining 2 groups, however, were not treated with any agent. After 1 hour, all the animals in groups 1, 2, 3, and 4 were challenged with 1ml of castor oil, orally, while the animals in the last group were left without castor oil to serve as normal control. All the animals were observed for consistency of faecal material (Awouters *et al.*, 1978). The faeces were collected in transparent plastic dishes placed beneath the individual rat cages.

Gastro-intestinal motility test

The rats for this study were divided into 4 groups (n = 4) and fasted for 18 hours before the experiment. The first group was left without any drug, group 2 received Atropine sulphate (3mg/kg, i. p.) and groups 3 and 4 received 50 and 100mg/kg doses of *P. guajava* leaf extract, respectively, 1 hour before the administration of 1ml castor oil. 1ml of marker (charcoal meal in acacia gum) was administered orally, 1 hour after castor oil treatment. The rats were sacrificed 1 hour later and the distance travelled by the charcoal meal from the pyrolus was measured as percentage of the total length of the intestine from the pyrolus to the caecum, that is % motility = Distance traveled by charcoal meal / Total length of the small intestine, as described by Mascolo *et al.*, 1994).

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the enteropooling assay as done by Robert *et al.*, (1976). Briefly, overnight (6pm – 9am) fasted rats were divided into 5 groups (n = 4). Groups 1 and 2 did not receive any treatment, while 3, 4, and 5 received 50 100mg/kg *P. guajava* leaf extract, orally, and 20mg/kg Chlopromazine (i. p.) respectively. Thirty minutes later, groups 2, 3, 4, and 5 were given 0.2ml castor oil p. o. The rats were later sacrificed thirty minutes after and the entire small intestines from each animal were weighed and their average weight per group was calculated.

The difference in the weight of the small intestine in control and castor oil treated groups was considered as the castor oil-induced accumulation of fluid (enteropooling).

Statistical Analysis

The experimental results were expressed as mean $\pm$ Satndard Error. Student- t – test was used for the evaluation of data and $p < 0.05$ accepted as significant.

RESULTS

Castor oil-induced Diarrhoea

Thirty minutes after administration of castor oil, the diarrhoea was clinically apparent in all the treated animals (Table 1). The frequency of diarrhoea was markedly reduced from an average 1.0 $\pm$ 0.4 to zero, by the intra-peritoneal injection of (3mg/kg) Dipheno-xylate. The aqueous extract showed a dose-dependent inhibition of castor oil induced diarrhoea just like the standard anti-diarrhoeal agent. At a dose of 50mg/kg, the extract reduced diarrhoea from an average of 4.5

± 0.29, in the first hour to zero in the fourth hour, while the 100mg/kg dose produced a reduction from a mean faecal frequency of 3.5 ± 0.29, first hour to zero in the fourth. The diarrhoea in the untreated control continued to rise from an average of 4.75 ± 0.85 in the first hour to 7.50 ± 0.29 in the fourth hour. Both the standard drug and the leaf extract showed significant ($p < 0.05$) reduction, when compared to the untreated control rats. In addition, both the treatments delayed the onset of diarrhoea and also reduced the wetness of the faecal droppings.

Small Intestinal Transit

The percentage intestinal transit distance was increased with castor oil (87.55%), but was reduced with each of the two (2) concentrations of the extract, and much markedly by Atropine sulphate (18.21%). The 50mg/kg and 100mg/kg doses of the extract produced a reduction (68.82% and 61.85%), of castor oil induced charcoal meal gastrointestinal transit (Table 2). There is a significant difference ($p < 0.05$) between the two treatments (*P. guajava* leaf extract and the standard drug) in reducing intestinal motility.

Table 1: Effects of leaf extract of *Psidium guajava* on castor oil induced diarrhoea

Treatment	**Hours after castor oil administration**			
	1*	**2**	**3**	**4**
Psidium (50mg/kg)	4.5 ± 0.29**	3.25± 0.48	2.5 ± 2.9	0[a]
Psidium(100mg/kg)	3.5 ± 0.29	2.75 ± 0.25	0	0[a]
Diphenoxylate(5mg/kg)	1.50 ± 0.4	1.25 ± 0.2	0	0[a]
Control (Castor oil alone)	4.75 ± 0.85	6.25 ± 0.75	8.75 ± 0.48	7.50 ± 0.29[b]
No castor oil, no extract	0	0	0	0

*= Hours after castor oil administration. ** = Mean defaecation in each group ± S. E
Note that means with the same superscripts are not significant ($p > 0.05$).

Table 2: Effects of aqueous leaf extract of *Psidium guajava* on gastro-intestinal transit distance

Treatment	Total length of intestine (cm)	Distance moved by marker (cm)	Intestinal transit distance (%)
Control(Castor oil alone)	60.25 ± 0.63	52. 75 ± 0.25[a]	87.55
CO + Psidium (50mg/kg)	65.75 ± 0.75	45.25 ± 0.25[b]	68.82
CO + Psidium (100mg/kg)	67.50 ± 0.65	41.75 ± 0.48[c]	61.85
CO + Atropine(5mg/kg)	72.75 ± 0.85	13.25 ± 0.29[d]	18.21

CO = Castor oil. Means with different subscripts are significant ($p > 0.05$).

Castor Oil Induced Enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop (Table 3). Castor oil-induced enteropooling is significantly inhibited by the reference drug Chlopromazine at a dose of 20mg/kg when compared to the control group. Each dose of the extract also produced a dose-dependent reduction in intestinal weight. 50mg/kg and 100mg/kg doses of extract produced 63.51 and 76.42% inhibition of weight of the intestinal contents respectively with significance ($p < 0.05$). There is no significant difference between the standard drug and the 100mg/kg concentration of the extract in their anti-enteropooling effects ($p > 0.05$).

Table 3: Anti-enteropooling effects of aqueous leaf extract of *Psidium guajava* in rats

Treatment	Weight of small intestine (g)	Castor oil-induced intestinal fluid (g)	Inhibition (%)
No CO, no extract	23.50 ± 0.65	……	……
Control(Castor oil alone)	39.75 ± 0.85	16.25	30.85
CO + Psidium (50mg/kg)	37.00 ± 0.82	13.50	42.55
CO + Psidium (100mg/kg)	30.75 ± 0.85	7.25	69.15
CO + Chlopromazine (5mg/kg)	28.50 ± 0.65	5.00	78.72

DISCUSSION

The results of the present study show that the leaf extract of *P. guajava* produced a significant reduction in the severity and frequency of diarrhoea produced by castor oil. It is also noted that the extract significantly reduced castor oil-induced intestinal transit distance. It also inhibited, dose-dependently, castor oil-induced intestinal fluid accumulation.

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in faeces (Horton *et al.*, 1968; Greenbergera *et al.*, 1978). Prostaglandins contribute to the patho-physiological functions of the gastro-intestinal tract and also act on the local electrical and mechanical activities of the ileo-caecal muscles (Sanders, 1984). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins, which stimulate motility and secretion (Pierce *et al.*, 1971).

Castor oil is reported to induced diarrhoea by increasing the volume of the intestinal content by preventing the re-absorption of water. The liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins which results in stimulation of secretion (Pierce *et al.*, 1971), thereby preventing the re-absorption NaCl and water (Galvez *et al.*, 1993).

The anti-diarrhoeal effect of the extract may probably be due to the fact that the extract have increased the re-absorption of NaCl and water by decreasing the intestinal motility as observed in the intestinal transit distance of charcoal meal. It may also be deduced that, since castor oil induced diarrhoea through elevated prostaglandins, the extract may act through the inhibition of prostaglandins synthesis and through reduction of propulsive movement of the small intestine.

The anti-diarrhoeal activity of the extract may also be due to the presence of denatured proteins forming tannates, which make the intestinal mucosa more resistant and hence reduce secretion (Tripathi, 1994). *P. guajava* is shown to possess tannins (Beckstrom-Stenberg, 1994).

The extract also inhibited castor oil-induced intestinal fluid accumulation (enteropooling). It has been shown that prostaglandins cause diarrhoea in experimental animals as well as human beings (Eakins and Sanner, 1972). Their mechanisms have been associated with dual effects on gastro-intestinal motility as well as water and electrolyte transport (Dajani *et al.*, 1975). This observation may be an indication that the extract (at doses of 50 and 100mg/kg) reduced diarrhoea by inhibiting castor oil-induced intestinal accumulation of fluid (Enteropooling).

Previous reports have demonstrated the anti-diarrhoeal activity of tannins (Mukherjee *et al.*, 1998), flavonoids (Galvez *et al.*, 1993), alkaloids (Gricilda *et al.*, 2001), saponins, reducing sugars and sterols and or terpenes (Otshudi *et al.*, 2000), containing plant extracts. The phyto-chemical analysis of *P. guajava* showed the presence of tannins, terpenes, flavonoids and saponins (Beckstrom-Stenberg, 1994).

The anti-diarrhoeal activity of flavonoids have been attributed to their ability to inhibit intestinal transit and hydro-electrolyte secretion (Di Carlo *et al.*, 1993; Galvez *et al.*, 1993; Rao *et al.*, 1997), which are known to be altered in the intestinal condition. Also, in vivo and in vitro experiments have shown that flavonoids are able to inhibit the intestinal secretory response, induced by prostaglandins (Sanchez and Medina *et al.*, 1997). In addition, flavonoids possess anti-oxidant properties (Su *et al.*, 2000) which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (Mora *et al.*, 1990). Previous research, in guinea pigs in Brazil, indicated guava leaf extracts to provide anti-oxidant effects beneficial to the heart. It also possesses heart protective properties and can improve myocardial function (Holetz *et al.*, 2002). As a result, it is possible to suggest that the anti-secretory and anti-oxidant properties of flavonoids could contribute to the anti-diarrhoeal effect.

In certain instances, it has been found that anti-diarrhoeal activity is associated with anti-microbial effect (Otshudi *et al.*, 2000). Earlier reports have shown the leaf extract of *P. guajava* to have *in vitro* toxic action against such common diarrhoea -causng bacteria as *Staphylococcus*, *Shigella*, *Salmonella*, *Bacillus*, *E. coli*, *Clostridium* and *Pseudomonas*. The leaf extract of guava plant is also shown to possess anti-fungal, anti-yeast (*Candida*), anti-amoebic and anti-plasmodial actions (Arima and Danno, 2002; Garcia *et al.*, 2002).

From these findings, it is therefore obvious that the aqueous leaf extract of *P. guajava* possesses significant anti-diarrhoeal activity due to its effect on both gastro-intestinal propulsion and fluid secretion. Since toxicity studies in rats, mice as well as controlled human studies show both the leaf and fruit extracts of guava plant to be safe and without side effects (Beckstrom-Stenberg, 1994), this means that guava leaf extract may perhaps provide a safe, cheap, available and effective treatment against *diarrhoea*. This will go a long way in alleviating the usual problems of health care delivery and at the same time atone the cultural yearnings of the local people, especially where dysentery, cholera and all *diarrhoea*-related disease are concerned.

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