

Original Article

Ameliorative Effects of Aqueous Extract of Avocado Pear Seed on Castor Oil Induced Diarrhea in Experimental Animals

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ABSTRACT

Diarrheal diseases affect billions of people globally and is the second leading cause of death in children under 5 years. Despite the efforts of governmental and non-governmental organizations to prevent or cure this disease, the burden of diarrheal diseases continues to be high. Therefore, there is need to further discover effective antidiarrheal drugs that are readily available and cheap to obtain with little or no side effects. Herbs and natural products have been used in the treatment of diarrhea; hence this study was carried out to investigate the effects of aqueous extract of avocado pear seed (AEAPS) on castor oil induced diarrhea in experimental animals. Fifty female Wistar rats weighing 100 – 120 g were used. The antidiarrheal effects of 200 mg/kg, 400 mg/kg and 600 mg/kg of AEAPS was carried out by investigating its effect on; faecal pellet output, castor oil induced diarrhea, intestinal enteropooling and intestinal transit time of charcoal meal. Result showed that 400mg/kg and 600 mg/kg AEAPS significantly ($P < 0.05$) reduced the number of faecal pellets from rats and the number of wet faecal pellets in castor oil induced diarrhea. The AEAPS at a dose of 400 mg/kg, 600 mg/kg and loperamide significantly ($P < 0.05$) inhibited intestinal enteropooling by 69.37%, 57.66% and 72.07% respectively. The treatments also significantly ($P < 0.05$) inhibited intestinal transit by 21.75%, 21.45% and 24.96% respectively relative to that in castor oil treated rats. The results suggest that AEAPS possess antidiarrheal effects that it exerts through antisecretory and antimotility mechanisms.

Keywords: Antisecretory, Antimotility, Avocado pear seed, Diarrhea

INTRODUCTION

Diarrheal diseases are among the leading causes of death globally; in the year 2017, about 1.6 million people died from diarrheal diseases globally. This is more than all deaths from all intentional injuries (suicide, homicide, in conflict and from terrorism) combined in the

same year.¹ Diarrheal diseases are the second leading cause of death among children under five years of age after pneumonia and it account for 9% of child deaths worldwide.² The World Health Organization (WHO) defines diarrhea as “the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual).³ Most diarrhea-associated morbidities and mortalities occur

in low- and medium-income countries, usually in rural areas as well as in the suburbs and slums of urban areas.^{4,5} These regions include Southern Asia and sub-Saharan Africa including Nigeria, where the prevalence is 18.8%.^{2,5,6} Deaths by diarrheal illness in developed nations are rare and account for about 4% of all hospital admissions.⁶ Diarrheal diseases are however both treatable and preventable. Vaccination against rotavirus, breastfeeding, safe water, improved hygiene and sanitation are key effective means of preventing significant morbidities and mortalities related to diarrheal disease.⁷ Treatment of the disease include the use of oral rehydration salt (ORS), zinc supplementation, rehydration with intravenous fluids, consumption of nutrient rich food and the use of antibiotics.³ In addition to the use of orthodox drugs, it has been claimed that several natural products and herbs can be used to treat diarrheal diseases.^{8,9,10,11} Many of these herbs and natural products are available, very assessable and may be cheap to purchase.

Avocado pear (*Persea Americana*) is a tree that belongs to the laurel family, *Lauraceae*. Avocado plant is indigenous to Central and South America, United States of America, Asia, parts of Europe, and Tropical Africa.¹² The avocado seed represents about 13-18% of total avocado fruit's weight and is an under-utilized resource that in most cases is thrown away.¹³ Phytochemical studies on avocado seeds have identified various classes of natural compounds such as phytosterols, triterpenes, fatty acids, furanoic acids, abscisic acid, proanthocyanidins, and polyphenols.¹⁴ It has been reported that extract of the seed of avocado pear seed possess cardioprotective, hypoglycaemic, fungicidal, larvicidal, hypolipidemic, amoebicidal, giardicidal and anticancer activities.^{12,15,16}

There are claims that avocado pear seed possess antidiarrheal activities, but this has not been scientifically proven.¹⁷ This research therefore aims to investigate the effects of aqueous extract of avocado pear seed on castor oil induced diarrhea in rats.

MATERIALS AND METHODS

Experimental Animals

Healthy female albino Wistar rats (100-120g) were purchased from the Animal House, College of Health Sciences, Benue State University, Makurdi and they were also housed within the same facility under standard controlled environmental conditions, with 12 hours light/dark cycle. They were fed with standard rat pellet purchased from Vital Feeds (Grand Cereal LTD, Jos,

Nigeria) and water *ad libitum*. Ethical clearance was sought and obtained for this study from the Ethical Committee, Benue State University, Makurdi. The animals were handled according to guideline of the Committee, which is in line with the internationally accepted principles for research on animals use.

The rats were housed in plastic cages, but for each experiment, wooden cages were used. They were constructed to accommodate only 1 rat, with gauze placed about 5 cm from the floor and white blotting paper was used to line the floor of the cages. Before the experiments, the rats were placed in these cages 2 h daily for one week, to make them familiar with the experimental environment.

Plant material and extraction

Ripe Avocado pears (*Persea Americana*) were purchased from Faringada Market, Jos, Plateau State Nigeria. The plant material was identified and authenticated by Mr. Joshua Waya, a Taxonomist in the Department of Botany, Faculty of Sciences, Benue State University, Makurdi. The succulent part of the fruit was removed to obtain the seeds. The seeds were minced using a grater and air dried before being ground to powder and stored in plastic container. The powder (200g) was soaked in distilled water (2 L) for 72h. After which it was filtered with Whatman No. 3 filter paper. The filtrate in a beaker was evaporated using a water bath set at 70°C. The concentrate was kept in the refrigerator before use.

Investigation of the effect of aqueous extract of avocado pear seed on faecal pellet output.

The rats were randomly divided into 4 groups and treated as follows: Group 1 was control that was given 1 mL/kg normal saline, Group 2 received 200 mg/kg AEAPS, Group 3, received 400 mg/kg AEAPS and Group 4 received 600 mg/kg AEAPS. All treatments were done orally. Faecal pellet count was done every hour and the final count was done after 5h of the treatments. The doses of AEAPS used in this study were in line with those used in previous studies.^{18,19}

Castor oil induced diarrhea

Thirty rats were divided into six groups of five rats each. The effects of AEAPS on castor oil induced diarrhea was done according to the method of Kola-Mustapha.¹⁰ The rats were fasted for 18 h. Group 1 was control, that received normal saline without further treatment. Animals in group 2 received normal saline, group 3 to 5 received 200 mg/kg, 400 mg/kg and 600 mg/kg of AEAPS respectively and

groups 6 was treated with 4 mg/kg loperamide. After 1 h, all animals received 1 mL of castor oil orally, except rats in group 1. The rats were placed in the experimental cages with white blotting paper below as described above and observed each hour for 4 h. The frequency of passage of watery (wet), unformed stool was counted. The normal saline + castor oil treated group result was considered as 100%. The activity of each group was expressed as percent inhibition (%) of diarrhea. The percentage inhibition of defecation was calculated as follows:

$$\% \text{ Inhibition of defecation} = \frac{A - B}{A} \times 100$$

where A indicates the mean number of defecations caused by castor oil and B indicates the mean number of defecations caused by plant extract.

Castor oil-induced enteropooling

In this method, as described by Adeniyi *et al.*,⁸ the rats were deprived of food for 18 h before the experiment. The rats were divided into five groups of five each. The vehicle (1 mL/kg normal saline) was given to the first group. Animal in groups 2, 3 and 4 received graded doses of AEAPS (200 mg/kg, 400 mg/kg and 600 mg/kg respectively), while animals in group 5 receive loperamide (4 mg/kg). Thirty minutes later, all the rats were treated with 1 mL of castor oil. One hour after castor oil administration, the rats were sacrificed; the ends of the small intestine were tied with thread before it was removed and weighed. The intestinal content was collected into a graduated cylinder, where its volume was measured. The intestine was then reweighed and the difference between the full and empty small intestine was calculated.

$$\text{inhibition (\%)} = \frac{W_{\text{control}} - W_{\text{treated}}}{W_{\text{control}}} \times 100$$

Where W_{control} refers to the weight of intestinal content in control group; W_{treated} refers to the weight of intestinal content in treated group.

Measurement of Intestinal Transit of Charcoal Meal

Assessment of the gastrointestinal propulsion of a charcoal meal was determined following the method of Tenorio *et al.*²⁰ The rats were fasted for 18 h, but had free access to water. At the end of the fast the animals were treated as described above. After 1 h of the treatments, they were fed 1 mL charcoal meal (10% charcoal suspension in 5% gum acacia) and at 30 mins after the meal, the animals were sacrificed, and the anterior abdominal wall was

immediately cut open to dissect out the whole small intestine (pylorus to caecum). The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured. This distance was expressed as a percentage of the length of the small intestine.

$$\text{Charcoal transit ratio (\%)} = \frac{\text{Total distance traveled by charcoal meal}}{\text{Total length of small intestine}} \times 100$$

Percentage inhibition of intestinal transit by treatment was calculated as:

$$\text{Inhibition (\%)} = \frac{(D_{\text{control}} - D_{\text{treated}})}{D_{\text{control}}} \times 100$$

Where D_{control} refers to the distance travelled in control group; D_{treated} refers to the distance travelled in treated group.

Statistical Analysis

Results were expressed as mean \pm SEM. Differences between the different groups were determined using One-way ANOVA with LSD post hoc test. The values were considered significant when $P < 0.05$. Data were analyzed using SPSS version 20.0 software (International Business Machines Corporation).

RESULTS

Effect of aqueous extract of avocado pear seed on faecal pellet output

Result showed that administration of aqueous extract of avocado pear seed (AEAPS) reduced the number of faecal pellet output in rats and this reduction was significant ($P < 0.05$) at a dose of 400 mg/kg and 600 mg/kg of the extract respectively as shown in Table 1.

Effect of aqueous extract of avocado pear seed on castor oil induced diarrhea.

Administration of castor oil resulted in significant increase in the frequency of defecation of wet faeces as shown in Table 2. Treatment with aqueous extract of avocado pear seed (200 mg/kg, 400 mg/kg and 600 mg/kg) significantly ($P < 0.05$) reduced the total number wet faecal pellet output and the percentage inhibition of defecation were 25.93%, 29.63% and 27.78% respectively, while 4 mg/kg loperamide exerted 33.33% inhibition. The decrease in

frequency of dropping wet faeces caused by the three doses of AEAPS were not significantly different from that caused by loperamide (Table 2).

Effect of aqueous extract of avocado pear seed on castor oil induced enteropooling.

Result showed that the three doses of aqueous extract of avocado pear seed used significantly ($P < 0.05$) reduced the

weight and volume of intestinal fluid and a dose of 400 mg/kg gave the most effective inhibition. The effect of the 400 mg/kg AEAPS on the weight and volume of intestinal fluid is not significantly different ($P > 0.05$) from that of loperamide (Table 3).

Effect of aqueous extract of avocado pear seed on

Table 1: Effect of aqueous extract of avocado pear seed on faecal pellet output

| Treatment | Number of faecal pellets |
|-----------------|--------------------------|
| Normal Saline | 9.8 ± 0.80 |
| 200 mg/kg AEAPS | 9.4 ± 0.83 |
| 400 mg/kg AEAPS | 6.0 ± 0.63* |
| 600 mg/kg AEAPS | 6.0 ± 1.92* |

Values expressed as mean ± SEM. * significant compared with control at $P < 0.05$

Table 2: Effect of aqueous extract of avocado pear seed on castor oil induced constipation

| Treatments | 1 hr | 2 hr | 3 hr | 4hr | Total | % inhibition |
|----------------------|-------------|-------------|-------------|-------------|---------------|--------------|
| Normal Saline | 1.20 ± 0.37 | 2.20 ± 0.37 | 3.00 ± 0.45 | 0.00 ± 0.00 | 6.40 ± 0.60 | |
| CO + Normal Saline | 8.40 ± 0.40 | 1.80 ± 0.20 | 0.20 ± 0.20 | 0.40 ± 0.24 | 10.80 ± 0.66* | |
| CO + 200 mg/kg AEAPS | 4.20 ± 1.07 | 2.00 ± 0.84 | 0.80 ± 0.49 | 1.00 ± 0.32 | 8.00 ± 0.45# | 25.93 |
| CO + 400 mg/kg AEAPS | 2.40 ± 0.87 | 2.40 ± 1.69 | 1.60 ± 0.40 | 1.20 ± 0.37 | 7.60 ± 2.16# | 29.63 |
| CO + 600 mg/kg AEAPS | 3.60 ± 0.75 | 3.20 ± 1.35 | 0.60 ± 0.40 | 0.40 ± 0.40 | 7.80 ± 1.74# | 27.78 |
| CO + Lop (4mg/kg) | 0.00 ± 0.00 | 4.80 ± 1.46 | 1.60 ± 0.51 | 0.80 ± 0.37 | 7.20 ± 1.83# | 33.33 |

Values presented as mean ± SEM. * significant compared with Normal saline treated at $P < 0.05$, # significant relative to CO + NS treated group at $P < 0.05$. CO = castor oil, AEAPS = aqueous extract of avocado pear seed. Lop = loperamide

Table 3: Effect of aqueous extract of avocado pear seed on castor oil induced enteropooling

| Treatments | Weight of intestinal content (g) | Inhibition rate in weight of intestinal content (%) | Volume of intestinal content (mL) |
|----------------------|----------------------------------|---|-----------------------------------|
| CO + NS | 2.22 ± 0.07 | | 2.98 ± 0.04 |
| CO + 200 mg/kg AEAPS | 1.22 ± 0.12*# | 45.05 | 0.94 ± 0.07*# |
| CO + 400 mg/kg AEAPS | 0.68 ± 1.39* | 69.37 | 0.60 ± 0.07* |
| CO + 600 mg/kg AEAPS | 0.94 ± 0.07*# | 57.66 | 0.74 ± 0.07*# |
| CO + Lop (4mg/kg) | 0.62 ± 0.04* | 72.07 | 0.44 ± 0.05* |

Values presented as mean ± SEM. * significant compared with normal saline treated at $P < 0.05$, # significant relative to CO + NS treated group at $P < 0.05$. CO = castor oil, NS = normal saline, AEAPS = aqueous extract of avocado pear seed. Lop = loperamide

Table 4: Effect of aqueous extract of avocado pear seed on gastrointestinal motility

| Treatments | Total length of intestine (cm) | Distance traveled by marker (cm) | Percentage of intestinal transit (%) | Inhibition rate (%) |
|----------------------|--------------------------------|----------------------------------|--------------------------------------|---------------------|
| CO + NS | 84.70 ± 3.08 | 56.20 ± 1.93 | 66.38 ± 0.43 | |
| CO + 200 mg/kg AEAPS | 71.80 ± 1.91 | 42.60 ± 1.33 | 59.32 ± 0.73*# | 10.64 |
| CO + 400 mg/kg AEAPS | 68.80 ± 3.60 | 35.80 ± 2.11 | 51.97 ± 0.87* | 21.71 |
| CO + 600 mg/kg AEAPS | 75.80 ± 2.28 | 39.60 ± 1.89 | 52.14 ± 1.08*# | 21.45 |
| CO + Lop (4mg/kg) | 68.80 ± 3.25 | 34.20 ± 1.32 | 49.81 ± 0.62* | 24.96 |

Values presented as mean ± SEM. * significant compared with normal saline treated at $P < 0.05$, # significant relative to CO + NS treated group at $P < 0.05$. CO = castor oil, NS = normal saline, AEAPS = aqueous extract of avocado pear seed. Lop = loperamide

gastrointestinal motility

Result showed that the percentage distance traveled by rats treated with castor oil + normal saline is $66.83 \pm 0.43\%$. Treatment with AEAPS (200mg/kg, 400 mg/kg and 600 mg/kg) significantly ($P < 0.05$) reduced the intestinal transit of charcoal meal, with 400 mg/kg of the extract producing the least distance travel ($51.97 \pm 0.87\%$). The percentage of intestinal transit of charcoal meal in loperamide treated is not significantly ($P > 0.05$) different from that of 400 mg/kg AEAPS (Table 4).

DISCUSSION

This study was carried out to investigate the antidiarrheal effects of aqueous extract of avocado pear seed in experimental animals. Result showed that AEAPS significantly reduced the frequency of diarrheal stools by decreasing the volume of intestinal contents and the intestinal secretions of the electrolytes and by reducing gastrointestinal peristalsis.

Castor oil was used in the study as previously used in other studies.^{8,9,10,11} Castor oil contains ricinoleic acid, which stimulates intestinal peristalsis, stimulates gastrointestinal secretion, inhibit water and electrolyte absorption, reduce active Na^+ and K^+ absorption and decrease Na^+ , K^+ -ATPase activity in the small intestine and colon.²¹ Ricinoleic acid also stimulates epithelial cells to produce nitric oxide and adenylate cyclase that lead to the production of prostaglandin, which induces diarrhea. Therefore, inhibition of prostaglandin biosynthesis is considered to inhibit ricinoleate-induced diarrhea.²²

Aqueous extract of avocado pear seed was found to possess the ability to inhibit enteropooling in castor oil-induced experimental animals by reducing both weight and volume of intestinal content. These effects are a result of reduced water and electrolytes secretion in small intestine. The extract may also enhance water and electrolyte absorption from intestinal lumen. Phytochemical screening revealed the AEAPS is rich in saponins, tannins, flavonoids and alkaloids.^{23,24} Antidiarrheal properties of medicinal plants were found to be due to tannins, flavonoids, alkaloids, saponins, reducing sugar, sterol and terpenes.^{11,25} Hence the saponin, alkaloids, tannins and flavonoids in AEAPS might be responsible for its antidiarrheal effects.

Aqueous extract of avocado pear seed significantly inhibited gastrointestinal transit of charcoal meal in rats. Diarrhea is characterized by increased peristaltic

movement, which rapidly removes the intestinal content. The AEAPS reduced the propulsive movement or gastrointestinal transit of charcoal meal, which indicates that extract might be capable of reducing the frequency of stools in diarrheal conditions. The reduction in gastrointestinal motility increases the time of stay of gastrointestinal contents in the intestine, and this may promote intestinal water and electrolyte absorption. This report of AEAPS on intestinal motility is similar to the report of several drugs and extracts that have antidiarrheal properties.^{8,9,11,26}

The antidiarrheal activities of AEAPS is similar to that of the standard drug loperamide. Loperamide is an opioid-receptor agonist that acts on the μ -opioid receptors in the mesenteric plexus of the large intestine; it decreases the activity of the mesenteric plexus, which in turn decreases the tone of the longitudinal and circular smooth muscles of the intestinal wall. Thus, intestinal transit time is slowed down.⁹ Further, loperamide inhibits intestinal secretion by acting on the δ receptor of the intestine.²⁷

CONCLUSION

The result of this study indicates that aqueous extract of avocado pear seed exerts antidiarrheal effects by inhibiting intestinal peristalsis and gastrointestinal secretion of water and electrolytes. These results provide a scientific basis for the use of the extract in the treatment of diarrhea.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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