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Evaluation of The Antidiarrheal Activity of Aqueous Stem Bark Extract of *Anogeissus leiocarpus* on Castor Oil-Induced Diarrhea in Rats

Maryam Usman Ahmed[1], Comfort Emmanuel[2], Yusuf Muhammad Adamu[3]
Adamawa State University, Mubi, Adamawa, Nigeria
maryam.usman@gmail.com

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Abstract

Diarrheal diseases are a major public health problem in developing countries. *Anogeissus leiocarpus* is used in Africa and particularly in Nigeria for the empirical treatment of diarrhea. The present study was undertaken to evaluate the effects of aqueous stem bark extract of *Anogeissus leiocarpus* on castor oil-induced diarrhea in rats. Thirty (30) Wistar rats of both sexes were grouped into six groups (the first three of which served as the normal control, negative control and standard treatment respectively, the last three were used as test groups) of five rats per group. Diarrhea was induced by administering 1 ml/rat of castor oil orally. Phytochemical screening of the aqueous stem bark extract of *Anogeissus leiocarpus* revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins and phenols. Treatment with the extract produced a significant dose-dependent inhibition of diarrhea. The percentage inhibition of diarrhea increased with corresponding increase in dose of the extract and was comparable with the percentage inhibition produced by the standard drug (loperamide). Treatment with this extract also produced a modest dose-dependent reduction on intestinal transit in rats and a statistically significant ($p<0.05$) dose-dependent reduction in the volume of intestinal content when compared with the negative control group. The results obtained from this

study suggests that the aqueous stem bark extract of *Anogeissus leiocarpus* have significant antidiarrheal effect on animal models and this finding supports the traditional use of the plant extract in the management of diarrhea.

Keywords: Diarrhea, antidiarrheal, Castor oil, Loperamide, Anogeissus leiocarpus

INTRODUCTION

Diarrhea is a disorder characterized by discharge of semisolid or watery fecal matter from the bowel three or more times in a day [1]. It involves an increase in the fluidity, volume and frequency of bowel movements, increased frequency of bowel sound, wet stools and abdominal pain, accompanied by increased secretion and decreased absorption of fluid, and thus loss of water and electrolytes [1]. This can progress to decreased urination, loss of skin color, a fast heart rate, and a decreases in responsiveness as it becomes more severe [2,6].

Diarrheal disease is the second leading cause of death among children below five (5) years globally. This accounts for about 9 percent of all deaths in 2012 [3]. This translates into about 1,600 children dying per day or about 580,000 children each year. These deaths mainly occur in south Asia and sub-Saharan Africa [4,5].

Diarrhea results when the delicate balance between the absorptive and secretory functions inside the intestine is impaired [7]. The major mechanisms that accounts for the pathophysiology of diarrhea are raised luminal osmolality, enhanced electrolyte secretion and reduced electrolyte absorption and accelerated intestinal motility [8]. To manage these symptoms, oral rehydration therapy is employed extensively. Treatment with oral rehydration therapy reduces dehydration resulting from diarrhea, It works as glucose increases the uptake of sodium and thus water by the intestines, and the potassium chloride and sodium citrate help prevent hypokalemia and acidosis, respectively, which are both common side effects of diarrhea [9,10,11]. Other treatment approach include the use of supplements (zinc sulfate, vitamin A, folate, magnesium, and copper), adsorbents (pectin, kaolin, activated charcoal, and lactobacillus sporogens), and other medications such as diphenoxylate, loperamide, bismuth sub- salicylate, codeine, lidamidine, and rececadotril

[12,13]. Gastrogard also serves as a prophylactic agent against diarrhea that is caused by retroviral infections in children [14].

Despite the fact that conventional therapies play significant roles in the management and or treatment of diarrhea, these medications still posed some challenges ranging from mild to serious side effects, some of which include constipation, headache, abdominal discomfort, nausea, dry mouth and vomiting [15]. It thus becomes important to identify, explore and evaluate new alternatives to currently available antidiarrheal drugs. One of these alternatives is the use of various parts of herbs in the form of infusion, decoction and enema [16]. Other methods include massage, acupressure and acupuncture [17].

Anogeissus leiocarpus is a deciduous tree species that can grow up to 15 – 18 m of height and up to 1 m diameter. The leaves are acute at the apex and attenuate at the base, pubescent beneath. It can reproduce by seeds as well as vegetative propagation [18]. *Anogeissus leiocarpus* is a typical element of woodlands and Savannas of the Sudanese regional center of endemism [19]. It belongs to the family Combretaceae, many secondary metabolites including flavonoids, tannins, phenolic acids, triterpenes, etc. have been isolated from this plant. It has a large ecological distribution ranging from the borders of Sahara up to the out layer humid tropical forests. *Anogeissus leiocarpus* is one of the several medicinal plants traditionally used for treatment of diarrhea [20]. This study endeavors to evaluate the antidiarrheal effects of the aqueous stem bark extract of *Anogeissus leiocarpus,* used in traditional medicine using castor oil induced diarrhea model.

MATERIALS AND METHODS

Collection and preparation of plant

Fresh stem bark of *Anogeissus leiocarpus* was collected from the Ecological Garden, Adamawa State University Mubi, Mubi North Local Government Area, Adamawa State. The plant was identified by a taxonomist in the Department of Botany, Adamawa State University, Mubi. The plant material was carefully and thoroughly washed using distilled water, thereafter, it was shade-dried at room temperature for seven days. The sample was pounded into powdered form using a sterilized mortar and pestle. The powdered sample was then macerated in distilled water for 72 h with occasional shaking using an orbital shaker. After maceration, this was then filtered using Whatman No. 1 filter paper. Excess

water was removed from the filtrate and evaporated to dryness in water bath at 50°C. The dried extract was stored in a sterilized polythene bag to be used when the need arises.

Preliminary phytochemical screening

The aqueous stem bark extract of *Anogeissus leiocarpus* was subjected to qualitative phytochemical screening according to the standard methods [21]

Experimental Animals

Wistar rats of both sexes weighing between 130 - 200 g were used in this experiment. The rats were housed and maintained under standard environmental conditions with free access to feed and tap water. Housing conditions and subsequent experiments were duly conducted with strict adherence to the guidelines established by the Institute of Laboratory Animal Research Commission on Life Sciences, National Research Council, Washington DC (1996), and approved by the University Ethical Committee, Adamawa State University, Mubi. Before the commencement of the experiment, the animals were acclimated to the laboratory conditions for two weeks.

Castor oil-induced diarrhea test in rats

The antidiarrheal activity of the aqueous stem bark extract of *Anogeissus leiorcarpus* was tested on castor oil induced diarrhea in Wistar rats according to the method described by Fokam *et al.* [22], with slight modifications. Thirty (30) rats were randomly divided into six groups; I, II, III, IV, V and VI (controls, standard treatment and three test groups) comprising of Five (n=5) rats per group. The animals were fasted for before the commencement of the experiment. Diarrhea was induced to groups II - VI by administering castor oil (1 ml/rat) to each rat. After diarrheal induction, each rat was placed individually in cages lined with filter paper. Thirty (30) minutes later, rats from group II received distilled water (10 ml/kg b. wt.) and served as diarrheal control group (DC), group III received the standard drug, loperamide (5 mg/kg), and the last three groups (groups IV, V and VI) served as test groups and received different doses orally (100, 200 and 400 mg/kg) of the extracts respectively using orogastric (feeding) tube. The filter papers were replaced on hourly basis for a period of four hours. The number of diarrheal stools over the four hours of observation was recorded. The Percentage Inhibition (I) of diarrhea in each group of treated rats were calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{Number\ of\ Diarrheal\ Stools\ in\ Control - Number\ of\ Diarrheal\ Stools\ in\ Test}{Number\ of\ Diarrheal\ Stools\ in\ Control} \times 100$$

Gastrointestinal motility test in rats

Another set of thirty (30) rats were randomly divided into six groups; I, II, III, IV, V and VI (controls, standard treatment and three test groups) comprising of Five (n=5) rats per group. The animals were fasted for 18 h before the commencement of the experiment. Diarrhea was induced to groups II - VI by administering castor oil (1 ml/rat). Thirty minutes after the induction of diarrhea, the rats in group II received normal saline (10 ml/kg). group III received loperamide (5 mg/kg) orally and the rats in the test groups (IV, V and VI) received orally, the aqueous extract of *Anogeissus leiocarpus*; 100, 200 and 400 mg/kg b. wt. respectively. Thirty (30) minutes after administration of these treatments, each rat received orally 1 ml of charcoal meal (5% activated charcoal by 5% gum acacia) as peristaltic marker. Thirty (30) minutes later the rats were sacrificed by cervical dislocation and the abdomen was opened using forceps. The intestine was removed, the total length of the small intestine (TLSI), and the distance covered by the charcoal meal in the small intestine (DCCM) were measured [23, 24]. The peristaltic index was calculated using the formula:

$$\text{P.I (\%)} = \frac{Distance\ Covered\ by\ Charcoal\ Meal}{Total\ Length\ of\ Small\ Intestine} \times 100$$

Castor oil-induced enteropooling test in rats

Another set of thirty (30) rats were randomly divided into six groups; I, II, III, IV, V and VI (controls, standard treatment and three test groups) comprising of Five (n=5) rats per group. The animals were fasted for 18h with free access to water before the commencement of the experiment. Diarrhea was induced to groups II - VI by administering castor oil (1 ml/rat). Thirty minutes after the induction of diarrhea, the rats in group II received normal saline (10 ml/kg). group III received loperamide (5 mg/kg) orally and the rats in the test groups (IV, V and VI) received orally, the aqueous extract of *Anogeissus leiocarpus*; 100, 200 and 400 mg/kg b. wt. respectively. One hour later, the rats were sacrificed by cervical dislocation, the abdomen was opened. The edges of the intestines (from pylorus to cecum) were tied with thread and the intestines were carefully removed. The contents of the intestines were collected by milking into a graduated tube and their volume measured [25]. The percentage inhibition of intestinal volume was calculated using the formula:

$$\text{I (\%)} = \frac{Volume\ of\ Intestinal\ Content\ in\ Control - Volume\ of\ Intestinal\ Content\ in\ Test}{Volume\ of\ Intestinal\ Content\ in\ Control} \times 100$$

Statistical analysis

Values were expressed as mean ± S.E.M (n=5). Statistical significance was determined by the one-way analysis of variance (ANOVA), followed by Turkey´s post hoc test, using the SPSS version 23, at $p<0.05$ level of significance.

RESULTS

Phytochemical screening

Table 1 shows the result obtained from the qualitative phytochemical screening of the aqueous stem bark extract of *Anogeissus leiocarpus*. This revealed the presence of alkaloids, flavonoids, tannins, steroids, saponins, and phenols.

Table 1: **Qualitative phytochemical screening of the aqueous stem bark extract of *Anogeissus leiocarpus***

Phytochemicals	Inference
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Saponins	+
Phenols	+

Key: + = present

Table 2 shows the effect of the aqueous stem bark extract of *Anogeissus leiocarpus* on stool inhibition of castor oil-induced diarrhea in rats. Thirty (30) minutes after the administration of castor oil, diarrhea was observed in all the groups with the exception of group I, to which castor oil was not administered. Upon treatment, reduction in the number of diarrheal stools was observed. The inhibition rate of diarrheal stools was significant across the standard treatment (loperamide) and the three test groups ($p<0.05$): 92.9%, 85.7%, 92.9% and 100.0% respectively (Table 2). The inhibition rate of the extract appears to be dose-dependent.

Table 2: **Effects of the aqueous stem bark extract of *Anogeissus leiocarpus* on stool inhibition in castor oil-induced diarrhea rats**

Groups	No. of diarrheal stools	% Inhibition
Group I (NS)	0.00 ± 0.00^{a}	-
Group II (NC)	14.00 ± 2.64^{c}	-
Group III (Lop5)	1.00 ± 0.00^{b}	92.9
Group IV (AlE100)	1.00 ± 0.50^{b}	85.7
Group V (AlE200)	1.00 ± 0.57^{b}	92.9
Group VI (AlE400)	0.00 ± 0.00^{a}	100.0

Values are expressed as Mean ± SEM.(n=5) Values with different superscript down the column indicates significant difference at $p<0.05$. NS: normal saline; NC: negative control; Lop5: loperamide 5 mg/kg; AlE100: *A.leiocarpus* extract 100 mg/kg; AlE200: *A.leiocarpus* extract 200 mg/kg; AlE400: *A.leiocarpus* extract 400 mg/kg.

Table 3 shows the effect of the aqueous stem bark extract of *Anogeissus leiocarpus* on intestinal motility in rats. The peristaltic index in normal control was 46.8%. Upon diarrheal induction, there was a significant increase as observed in the negative control (57.3%). The standard drug loperamide (5 mg/kg) and the aqueous stem bark extract of *Anogeissus leiocarpus* (100, 200 and 400 mg/kg b. wt.) significantly ($p<0.05$) reduced this effect (25.9%, 41.0%, 34.6% and 33.3% respectively) as shown in table 3.

Table 3**: Effect of the aqueous stem bark extract of *Anogeissus leiocarpus* on intestinal motility in castor oil induced diarrheal rats**

Groups	TLSI	DTCM	PI(%)
Group I (NS)	89.67 ± 1.20	42.00 ± 0.57	46.8
Group II (NC)	85.00 ± 0.57	48.67 ± 0.33	57.3
Group III (Lop5)	82.33 ± 2.40	21.33 ± 1.20	25.9
Group IV (AlE100)	85.33 ± 0.88	35.00 ± 1.00	41.0
Group V (AlE200)	91.67 ± 1.76	31.67 ± 3.38	34.6
Group VI (AlE400)	92.00 ± 1.15	30.67 ± 1.76	33.3

Values are expressed as Mean ± SEM.(n=5) Values with different superscript down the column indicates significant difference at $p<0.05$. NS: normal saline; NC: negative control; Lop5: loperamide 5 mg/kg; AlE100: *A.leiocarpus* extract 100 mg/kg; AlE200: *A.leiocarpus* extract 200 mg/kg; AlE400: *A.leiocarpus* extract 400 mg/kg; TLSI: total length of small intestine; DCCM: distance covered by charcoal meal; PI: peristaltic index.

Table 4 shows the effect of the aqueous stem bark extract of *Anogeissus leiocarpus* on castor oil-induced enteropooling in diarrheal rats. Castor oil induced the accumulation of water and electrolytes in the intestinal loop. This is evident from the increased intestinal volume content observed in the negative control group (group II). However, treatment with the standard drug (loperamode 5 mg/kg) and different doses of the extract, caused a significant decrease in the volume of the intestinal content across the treated groups (III, IV, V, VI), as the dosage increases, the volume of the intestinal content decreases.

Table 4**: Effect of the stem bark extract of *Anogeissus leiocarpus* on castor oil-induced enteropooling in diarrheal rats**

Groups	**Volume of Intestinal Content**	**%**
Inhibition		
Group I (NS)	1.33 ± 0.57^{b}	-
Group II (NC)	3.06 ± 0.40^{d}	-
Group III (Lop5)	0.46 ± 0.05^{a}	85.0
Group IV (AlE100)	2.63 ± 1.18^{bc}	14.1
Group V (AlE200)	1.43 ± 0.23^{b}	53.3
Group VI (AlE400)	1.13 ± 0.11^{b}	63.1

Values are expressed as Mean ± SEM.(n=5) Values with different superscript down the column indicates significant difference at $p<0.05$. NS: normal saline; NC: negative control; Lop5: loperamide 5 mg/kg; AlE100: *A.leiocarpus* extract 100 mg/kg; AlE200: *A.leiocarpus* extract 200 mg/kg; AlE400: *A.leiocarpus* extract 400 mg/kg.

DISCUSSION

The phytochemical screening of the aqueous stem bark extract of *Anogeissus leiocarpus* revealed the presence of alkaloids, tannins, steroids, flavonoids, saponins and phenols. The antidiarrheal activity of many plants have been found to be due to the presence of tannins, alkaloids, flavonoids and terpenoids [26]. These phytochemicals exert antidiarrheal activity through various mechanisms. Tannins and flavonoids increase caloric water and electrolyte reabsorption. Flavonoids and terpenoids inhibits the release of ricinoleic acid and prostaglandins [27].

The action of castor oil as a diarrhea inductor has been largely studied, and its active component is ricinoleic acid, which produces an irritating and inflammatory action on the

intestinal mucosa, leading to the release of prostaglandins. This condition increases the permeability of the mucosal cells and provokes changes in electrolyte transport thus causing diarrhea [28].

In this study, there was a statistically significant reduction in the number of diarrheal stools produced in the treatment groups. All the tested doses of the extract significantly reduced the total diarrheal stools ($p < 0.05$) compared with the negative control group. The percentage inhibition of diarrhea increases with corresponding increase in dosage of the plant extract, and was comparable with the percentage inhibition produced by the standard drug, thus, it is possible that the extract reduced the number of diarrheal stools by increasing reabsorption of electrolytes and water or by inhibiting the induced accumulation of fluid just as the standard drug, loperamide.

Treatment with this extract also produced a modest dose-dependent reduction on intestinal transit in rats and a statistically significant ($p<0.05$) dose-dependent reduction in the volume of intestinal content when compared with the negative control group.

The result obtained from this study agrees with reports elsewhere, where the stem bark extracts of *Anogeissus leiocarpus* [29] produces a significant inhibition on castor oil-induced diarrhea in rats.

The antidiarrheal activity of this plant extract may be justified by the presence of compounds such as flavonoids, alkaloids and tannins [26, 27].

CONCLUSION

The results obtained from this study shows that the ethanol stem bark extract of *Anogeissus leiocarpus* contains some pharmacologically active substances with antidiarrheal properties. The result of this study might provide the scientific evidence for the traditional use of this plant extract as an antidiarrheal remedy.

Conflicts of interest

No conflict of interest declared by the authors

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