Effects of Aqueous Stem Bark Extract of *Citrus aurantifolia* on the Gastrointestinal Tract of Wistar Rats

Abstract

Objective: The anti-diarrhea activity of the aqueous extract of the stem bark of *C. aurantifolia* was investigated in this study.
Materials and Methods: Acute toxicity evaluation of the aqueous extract of *C. aurantifolia* stem bark was carried out using rats according to Lorke's method. Experimental diarrhea was induced in rats with castor oil, and the effect of the extract on castor oil-induced gastrointestinal motility and enteropooling was consequently investigated.
Results: In the acute toxicity study, the extract produced no signs of toxicity or mortality in rats up to a dose of 5000 mg/kg. The oral LD_{50} of the aqueous stem bark extract of *C. aurantifolia* was therefore taken to be >5000mg /kg. The extract significantly ($p<0.05$) decreased the frequency of defecation as well as in the number of unformed faeces produced by castor oil-induced diarrhea in a dose- dependent manner. The extract also decreased the distance travelled by activated charcoal in the gastrointestinal tract of treated rats when compared to control rats. Results of castor oil-induced enteropooling revealed slight reduction in the weight of intestinal contents of treated rats compared to control rats.
Conclusion: This study therefore, clearly shows that *Citrus aurantifolia* stem bark possess significant anti-antidiarrheal potential and could be useful in the treatment of diarrhea.

Key words: Gastrointestinal tract, Aqueous, Stem Bark, *Citrus aurantifolia*, Wistar rats

1.0 INTRODUCTION

Diarrhea can be defined as an alteration in the normal bowel movement, characterized by a situation in which an adult's daily stools exceeds 300 g and contains 60 – 95 % water [1]. The WHO estimation revealed that diarrhea causes 45 million deaths annually throughout the world. 80% of these deaths are reported in developing countries including Nigeria. In Nigeria, diarrheal infection remains the number one killer disease among children under 5 years, while 7- 12 month old babies remain the most susceptible [2]. Acute diarrhea being the most common is usually caused by an infectious agent, even though drugs, poisons or acute inflammatory reactions are contributing factors [3]. Rotavirus is the major causative agent of infectious diarrhea, particularly

in young children Nowadays, however, other viral (*Enterovirus, norovirus* and adenovirus), bacterial (*Salmonella* sp., *Shigella* sp., *Escherichia coli*, *Camphylobacter* and *Vibrio cholerae*) and parasitic (*Cryptosporidium* and *Giardia*) agents are important pathogens [4].

Despite the effective and simple cheap treatment of oral rehydration therapy, majority of the local populace still rely on herbs to treat diarrhea [5]. The use of herbal drugs in the treatment of diarrhea is a common practice in many developing countries; here we attempt to investigate the folklore claim of *Citrus aurantifolia* stem bark extract for antidiarrheal activity. *Citrus aurantifolia* belongs to the family Rutaceae (orange family) [6]. *Citrus aurantifolia* is a Perennial Tree, with evergreen leaves, thorny stem, whitish flowers, globase fruits with many seeds, green when unripe and greenish yellow when ripe, with sour taste [7]. *C. aurantifolia* is used in the folklore medicine as an antiseptic, anthelmintic, mosquito bite repellent, for stomach ailments, tonic, antiscorbutic, astringent, diuretic, headache, arthritis, digestive and appetite stimulant, and for colds, coughs and sore throats [8], [9]. *C. aurantifolia* alleviates anxiety and nervousness, relieves stress related disorders such as insomnia or nervous originated digestive disorders, and also possesses anti-inflammatory potential/ an anticoagulant property, which renders it very valuable for people with cardiovascular risks. It is also used against fever, headaches and cold [10].

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and drugs

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor while the drugs were purchased from a local pharmacy shop.

2.1.2 Animals

Adult Wistar rats of either sex weighing 150–200g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

2.2 Methods

2.2.1 Plant Collection and Identification

The stem bark of *Citrus aurantifolia* was collected from a natural habitat in Agbeji Area of Kogi State, Nigeria. The plants were identified and authenticated at the herbarium unit of Biological Sciences Department, Federal University, Lokoja.

2.2.2 Preparation of Extracts

The plant material was shade- dried for twenty one (21) days and blended. One thousand and five hundred (1500) gram of the blended stem bark was soaked in distilled water for 72 hours. The resulting mixture was filtered using Whatmann filter paper (Size No1) and the extract was concentrated using a free- dryer. The extract was labelled as Aqueous stem extract of *Citrus aurantifolia* (SECA).

2.2.3 Acute Toxicity Study

The oral median lethal dose (LD_{50}) of the extract was determined in rats according to the method of Lorke [11].

2.2.4 Experimental Design

2.2.4.1 Castor oil-induced diarrhea

The method of Offiah and Chikwendu [12] was adopted. Twenty- five (25) rats of both sexes were fasted overnight but allowed free access to water. They were randomized into five groups of five rats each. Group I served as control and were administered 2 ml normal saline (0.9%). Groups II- IV were administered 125, 250 and 500 mg/kg of SECA orally while Group V was administered diphenoxylate hydrochloride (5 mg/kg) intraperitoneally. All rats were housed singly in a cage lined with white blotting paper. One hour after treatments, each of the rats was treated with 1 ml of castor oil orally. Rats were then observed for 6 hours and the number of spots with watery faeces on the white bloated paper lining the cage where individual rat was kept. Percentage protection was calculated as follows

Mean number of defecation of control- Mean number of treated group

$$\% \text{ Protection} = \frac{\text{Mean number of defecation of control}}{\text{Mean number of defecation of control}} \times 100$$

2.2.4.2 Effect of castor oil-induced gastrointestinal motility

The method of Chitme et al. [13] was adopted. Rats were fasted overnight and then randomized into five groups of five rats each and allowed free access to water. Group I served as control and was administered 2 ml normal saline (0.9%) orally while group V was administered 3 mg/kg of atropine intraperitoneally. Groups II-IV was administered 125, 250 mg/kg, and 500 mg/kg of the SECA orally. After 10 min of administering the extract and drug, 1 ml of 5% activated charcoal suspension in 10% aqueous solution of Acacia powder was administered to treated rats. Rats were then sacrificed 30 minutes later and the abdomen was opened to measure the distance travelled by the activated charcoal. The results were expressed as percentage of the total length of the intestine from the pylorus to the caecum.

2.2.4.3 Effect of castor oil-induced enteropooling

The method of Robert et al. [14] was adopted. The intraluminal fluid accumulation due to the effect of castor oil was determined. Rats were fasted overnight but allowed access to fresh drinking water. The rats were randomized into five groups of five rats each. Group I served as control and was administered 2 ml normal saline. Groups II- IV were administered 125, 250 and 500 mg/kg of SECA orally. Group V was administered atropine (3 mg/kg) intraperitoneally. An hour later, 1 ml of castor oil was administered to each of the treated rats. They were then sacrificed after 1hour post castor oil administration. The small intestines were removed, tied at both ends with thread and weighed. Intestinal contents were collected by milking and the volume measured.

2.2.5 Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Fishers test and difference between means at $P< 0.05$ were considered significant.

3.0 RESULTS

3.1 Acute Toxicity

The results of acute toxicity studies showed no mortality or signs of toxicity up to a dose of 5000 mg/kg of aqueous extract of *Citrus aurantifolia.* The oral LD_{50} of the extract was therefore taken to be > 5000 mg/kg (Table 1).

Table 1: Effect of Aqueous Stem Bark Extract *Citrus aurantifolia* (SECA) on Rats in Acute Toxicity Study

Phase of Study	Dose (mg/ kg)	Signs of Toxicity	Mortality/ n
I	10	-	0/3
	100	-	0/3
	1000	-	0/3
II	1600	-	0/1
	2900	-	0/1
	5000	-	0/1

Note, n = number of rats treated

3.2 Effect of Aqueous Stem Bark Extract *Citrus aurantifolia* (SECA) on Castor Oil-induced Diarrhea in Albino Rats

Table 2 shows the frequency of defecation by the rats within 6 hours of administration of SECA and castor oil. There was a significant ($p<0.05$) difference in the frequency of defecation between the control group and treated groups. The extract showed a dose- dependent effect as the group treated with 500 mg/kg SECA had the lowest frequency of defecation and the highest percentage of inhibition (65.01%) followed by the 250 and 125 mg/kg SECA- treated groups with 59.06 and 53.25% of inhibition respectively. There was no significant ($p>0.05$) difference in percentage inhibition between group V (standard drug- diphenoxylate hydrochloride –treated rats) and the groups admitted SECA at all doses.

Table 2: Effect of Aqueous Stem Bark Extract *Citrus aurantifolia* (SECA) on Castor Oil-induced Diarrhea in Albino Rats

Group	Treatment (mg/kg)	Mean number of Defecation (6 hrs)	Percentage Protection (%)
I	Control	7.23±1.14	-
II	SECA 125mg/ kg+ CO	3.38±1.01[a]	53.25
III	SECA 250mg/ kg+ CO	2.96±0.96[a]	59.06
IV	SECA 500mg/ kg+ CO	2.53±0.55[a]	65.01
V	Diphynoxylate + CO	2.22±0.47[a]	69.30

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Fisher's test, (n=5). [a] Statistically significant at $p < 0.05$. CO= Castor oil

3.3 Effect of Aqueous Stem Bark Extract of *Citrus aurantifolia* (SECA) on Gastrointestinal Motility

The effect of the extract on gastrointestinal transit of activated charcoal is shown in Table 3. There was a significant ($p<0.05$) decrease in the intestinal transit of activated charcoal in SECA-treated groups compared to the control group. The charcoal travelled very rapidly in the control group while the rate of movement was significantly ($p<0.05$) reduced in rats treated with SECA in a dose- dependent manner. The 500 mg/kg SECA –treated rats had a charcoal movement rate comparable to the 3 mg/kg atropine- group. The transit of charcoal in the groups treated with 125 and 250 mg/kg SECA were also statistically similar to the atropine- treated group.

Table 3: Effect of Aqueous Stem Bark Extract of *Citrus aurantifolia* (SECA) on Charcoal Gastrointestinal Transit in Albino Rats

Group	Treatment (mg/kg)	Length of Intestine (cm)	Distance Travelled by Charcoal (cm)	Percent Intestinal Transit (%)
I	Control	35.2±1.14	30.7±3.21	87.22
II	SECA 125mg/ kg+ Ch	33.4±1.18	20.8±2.12	62.28[a]

III	SECA 250mg/ kg+ Ch	34.6±2.33	18.3±2.33	52.89[a]
IV	SECA 500mg/ kg+ Ch	34.1±1.43	16.1±1.98	47.21[a]
V	Atropine 3mg/kg + Ch	36.4±2.17	16.6±1.56	45.60[a]

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Fisher's test, (n=5). [a] Statistically significant at $p< 0.05$. Ch = charcoal

3.4 Effect of Aqueous Stem Bark Extract of *Citrus aurantifolia* (SECA) on Castor oil-Induced Enteropooling

The effect of the extract on castor oil-induced enteropooling is shown in Table 4. The result showed that there was a significant decrease ($p<0.05$) between the volume of intestinal contents in the control group and the treated groups. The volume of fluid in the group treated with 500 mg/kg of SECA had comparable result to that of atropine-treated group. The 125 mg/kg treated group had the highest percentage intestinal fluid inhibition of 49.01% followed by the group treated with 250 mg/kg of SECA while the group treated with 500 mg/kg had the least percentage intestinal fluid inhibition.

Table 4: Effect of Aqueous Stem Bark Extract of *Citrus aurantifolia* (SECA) on Castor oil-induced Diarrhea in Albino Rats

Group	Treatment (mg/kg)	Wt. of Full Intestine (g)	Wt. of Empty Intestine (g)	Wt. of Intestinal Content (g)	Percentage Inhibition of Fluid (%)
I	Control	4.32±0.58	1.79±0.45	2.53±0.11	-
II	SECA 125mg/ kg+ Ch	4.10±0.73	2.81±0.34	1.29±0.01[a]	49.01
III	SECA 250mg/ kg+ Ch	4.12±0.69	2.61±0.39	1.51±0.23[a]	40.32
IV	SECA 500mg/ kg+ Ch	4.08±0.52	1.94±0.45	2.14±0.23[a]	15.42
V	Atropine 3mg/kg + Ch	3.99±0.48	1.88±0.43	2.11±0.31[a]	16.60

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Fisher's test, (n=5). [a] Statistically significant at $p< 0.05$. Ch = Charcoal

4.0 DISCUSSION

In Table 1, acute toxicity of the extract revealed that oral administration of the extract up to a dose of 5000mg/ kg produced no immediate signs of toxicity or mortality. The LD_{50} of the extract was therefore estimated to be above 5000 mg/kg according to Lorke's method [11]. This implies that the extract can be administered with some degree of safety, especially through oral route, where absorption might not be complete due to inherent factors limiting gastrointestinal tract absorption.

The aqueous stem bark extract of *Citrus aurantifolia* exhibited a dose-dependent protective effect against diarrhea (table 2). Diarrhea induced by castor oil results from the action of ricinoleic acid which causes the irritation and inflammation of the intestinal mucosa leading to prostaglandins (PGE2α) release. The released PGE2 stimulates gastrointestinal motility and secretion of water and electrolytes [15], thus inducing an increase in the peristalsis and an intestinal hyper secretion of fluid. The inhibition of prostaglandins biosynthesis prolongs the time of induction of diarrhea by castor oil [16]. In addition to the increase in the latency time and a decrease in the frequency of defecation, the administration of SECA to rats also caused a significant reduction of total fresh weight of deposit, of water content and of the surface of impregnation of deposit. These results are similar to those obtained with diphenoxylate used as standard drug and suggest that SECA might act as diphenoxylate. In fact, the antidiarrheal activity of diphenoxylate results from its antispasmogenic and antisecretory properties on the intestine [17].

Study also showed that *C. aurantifolia* significantly produced a significant reduction in the progression of charcoal meal and in the intestinal transit time dose- dependently (table 3). The 500 mg/ kg SECA produced a reduction comparable to that of atropine used here as reference drug and which is known to reduce intestinal motility [18]. Since the extract has demonstrated the ability to inhibit castor oil-induced diarrhea, its anti-diarrheic effect might in part be due to decreased gastrointestinal secretion and/or inhibition of gastrointestinal motility. The decreased intestinal motility and intestinal charcoal transit time might be due to increased re-absorption of water as earlier reported by Sahoo *et al*. [19].

The extract also produced a reduction of castor oil-induced enteropooling in a dose- dependent manner as shown in table 4. This observation might be due to the ability of the extract to mediate a reduction in weight gain of intestinal contents by preventing fluid and electrolyte secretion into the intestine through the reduction of gastrointestinal motility. This is because reduction of the gastrointestinal motility normally allows intestinal content ample time to be exposed to the absorptive surface of the intestinal tract [20]. Diphenoxylate hydrochloride, an opioid, is known to inhibit gastrointestinal secretions and motility, as exhibited by the study extract. Therefore, it could be inferred from the study that the decrease in frequency of defecation and distance travelled by the charcoal meal might be due to the inhibition of the gastrointestinal motility by the extract. It can also be suggested that effects of the extract might be mediated through α-2 adrenergic receptor stimulation.

5.0 Conclusions

The aqueous extract of *Citrus aurantifolia* stem bark exhibited a dose-dependent effect against diarrhea; it significantly inhibited castor oil-induced intestinal fluid accumulation and the volume of intestinal content and also significantly ($p < 0.05$) reduced the castor oil induced intestinal transit. This study therefore, clearly shows that *citrus aurantifolia stem bark* possess significant anti-antidiarrheal potential and could be useful in the treatment of diarrhea.

Ethical Disclaimer:

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

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