

Laxative Effects of Aqueous Extract of *Cida acuta* Leaves in Loperamide-induced Constipation in Wistar Rats

Abstract

Aim: To investigate the potentials of aqueous leaf extract of *Cida acuta* in loperamide-induced constipation in Wistar rats.

Methods: Constipation was induced by oral administration of loperamide (3 mg/kg b.wt.). The constipated rats were orally treated daily either with 200, 400, 800 mg/kg body weight per day of the extract or 0.21 mg/kg bisacodyl (reference drug) for 7 days while the normal and constipated control groups received distilled water. The feeding characteristics, body weight, faecal properties and gastrointestinal transit ratio were monitored throughout the study period.

Results: There was significant decrease ($p < 0.05$) between normal and constipated rats in the number of faecal pellets and water content of faecal pellets while there was no significant changes in the feed/ water intake and body weight of rats. Administration of the graded doses of the extract to the constipated rats significantly and dose- dependently normalized ($p < 0.05$) the number of faecal pellets/ water content of faecal pellets and gastrointestinal ratio compared to the constipated control..

Conclusion: The aqueous root extract of *Cida acuta* possesses laxative activity in loperamide- induced constipated rats.

Keywords: Constipation, *Cida acuta*, Loperamide, Laxative, Bisacodyl

1.0 INTRODUCTION

Constipation is a common problem worldwide and contributes significantly to health care financial burden. The worldwide prevalence of functional constipation varies from 0.7% to 29.6% in children and from 2% to 35% in adults depending on the geographical region ^{[1], [2], [3]}. Constipation significantly affects quality of life as it can cause not only discomfort and restlessness but also abdominal distension, vomiting, gut obstruction, and perforation, and it is even associated with fatal pulmonary embolism ^[4]. As a majority of laxative drugs have side effects, it is worthwhile to search for medicinal plants with laxative effects.

Traditional knowledge to solve health problems of mankind and animals exists in all countries of the world, with history dating back to as long as 3000 BC years ago ^{[5], [6]}. In most of the traditional medicine, the medicinal plants include the fresh or dried part, whole, chopped or powdered. Many medicinal plants have been used locally for the treatment of constipation and one of such plants is *Cida acuta*. Hence, this study was undertaken to investigate the laxative activity of the aqueous leaf extract of *Cida acuta* in loperamide-induced constipated rats. This would provide the scientific backing for its use as a laxative.

Sida acuta is a small erect, much branched, perennial shrub or herb, ranging from 30 to 100cm in height with a strong tap root; stem and branches flattened at the extremities, fibrous, almost woody at times, leaves alternate, slender, lanceolate, acute, margins toothed, 1.2 to 9cm or longer, 0.5 to 4cm wide, lower surface smooth or with sparse, short branched star-like (Stellate) hairs with fairly prominent veins; petiole 3 to 6mm long, hairy with a pair of stipules at least one lanceolate- linear 1 to 2mm broad, three to six nerved, often curved, finally hairy, the other stipule narrower, one to four nerved. The medicinal values of *Sida acuta* plant lies in some chemical substances that produce definite physiological actions on the human body. Some of these bioactive constituents of plants are classified as alkaloids, tannins, flavonoids, saponins, phenolic compounds; and other compounds reported to possess diverse range of bioactivity^{[7], [8]}. All parts of the plant are used for therapeutic purposes, but the leaves are the most frequently request. Leaves are considered to possess demulcent, diuretic, anthelmintic and wound healing properties, and are used to treat rheumatic affections^{[9], [10]}. The leaves decoction is used to treat abdominal pain, hemorrhoids, azoospermia and oligospermia^[11]. The leaf juice is also used in India for vomiting and gastric disorders^[12]. The roots of the *Sida* species are considered excellent adaptogenic and immunomodulator, general nutritive tonic and prolonged life; useful in tuberculosis and in diseases associated with injury, heart diseases, cough and respiratory diseases^[13].

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and drugs

Loperamide hydrochloride, carboxymethylcellulose (products of BDH Chemicals Ltd. Poole, England) and Bisacodyl (Medrel Pharmaceuticals Ltd, India) where purchased locally from local vendors.

2.1.2 Animals

Adult Wistar rats of either sex weighing 180–250g 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 leaves of *Cida acuta* were collected from a natural habitat in Auchi Area of Edo State, Nigeria. The plants were identified at the Herbarium Unit of the Department of Biology,

Ambrose Alli University, Ekpoma, Nigeria and voucher specimens were deposited for future references.

2.2.2 Preparation of Extracts

The leaves of *Cida acuta* were shade- dried for seven (5) days and pulverized using an electric blender. Two thousand (3000) gram of the pulverized leaves was soaked in distilled water for 96- hours. The resulting mixture was filtered using Whatmann filter paper (Size No1) and the extract (referred to as CAAE henceforth) was concentrated using a free- dryer.

2.2.3 Acute Toxicity Study

The oral median lethal dose (LD₅₀) of the extract was determined in rats according to the method of Lorke ^[14].

2.2.4 Experimental Design

2.2.4.1 Induction of constipation

Constipation was induced in the animals by the oral administration of loperamide (3 mg/kg body weight daily for 3 days) ^[15], while the control rats were administered with distilled water only. The passage of reduced, hard and dry fecal pellets indicated constipation in the rats on the 4th day.

2.2.4.2 Grouping and treatment of constipated rats

The rats were divided into six groups of five rats each. The animals in Group 1 (control) and Group 2 (constipated control) were administered with 1ml distilled water orally. Groups 3, 4 and 5 comprised constipated rats administered 200, 400 and 800 mg/kg body weight per day of leaf- extract of *Cida acuta* respectively while Group 6 were constipated rats administered bisacodyl (0.21 mg/kg body weight) ^[16]. All oral administration was done using metal oropharyngeal cannula. The water and feed intake, number of faecal pellets and body weight gain of all the rats were recorded during experimental period.

2.2.4.3 Determination of total number, dry weight and water content of faecal pellets

The excreted faecal pellets of individual rats were collected every day at 10.00 h throughout the duration of the experiment for 7 days. The total number, weight and water content of the pellets were determined. The water content was calculated as the difference between the wet and dry weights of the pellet ^[15].

2.2.4.4 Determination of gastrointestinal transit (GIT) Ratio

GIT ratio was measured according to the method described by ^[15]. On the 7th day, 1 ml of carmine (3 g suspended in 50 ml of 0.5 % carboxymethylcellulose) was orally administered to the rats. One hour after administering carmine, the animals were sacrificed and their small intestines were quickly removed. The distance over which the carmine had travelled and the total length of the small intestine were measured. The GIT ratio was expressed as the percentage of the distance travelled by the carmine relative to the total length of the small intestine.

2.2.5 Statistical Analysis

Data were expressed as means \pm SEM of four replicates and were subjected to one way analysis of variance (ANOVA) followed by Bonferroni tests to determine significant differences in all the parameters. All data were analysed using GraphPad Prism 5 statistical package (GraphPad Software, San Diego MA, USA) and values were considered statistically significant at $p < 0.05$.

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 *Cida acuta*. The oral LD₅₀ of the extract was then taken to be > 5000 mg/kg.

3.2 Effect of Loperamide on Feed and Water Intake, Fecal Properties and Body Weight of Constipated Rats before Treatment

Table 1 shows the effect of Loperamide on feed and water intake, fecal properties and body weight of constipated rats before treatment. Loperamide significantly ($p < 0.05$) reduced the number of faecal pellets and water content of faecal pellets of rats compared to the normal rats. This was an indication of constipation. However, there was no significant difference ($p > 0.05$) between feed intake, water intake and body weight of the constipated animals compared to the normal rats.

Table 1: Effect of Loperamide on Feed and Water Intake, Fecal Properties and Body Weight of Constipated Rats before Treatment

Parameters	Normal rats	Constipated rats
Feed intake (g)	17.31±1.31	15.11±0.91
Water intake (ml)	26.15±0.80	25.48±1.63
Number of faecal pellets	60.23±2.61	47.23±2.15 [*]
Water content of faecal pellets (ml)	3.25±0.12	1.23±0.09 [*]
Weight of rats (g)	156.48±10.23	159.35±11.43

Data are presented as mean ± SD. (n=5); * significantly different from normal control at p< 0.05

3.3 Effect of Aqueous Extract of *Cida acuta* on Feed and Water Intake, Body Weight Gain and Faecal Properties of Constipated Rats after Treatment

Table 2 shows the effect of aqueous extract of *Cida acuta* on feed and water intake, body weight gain and faecal properties of constipated rats after treatment. There was no significant difference ($p > 0.05$) in the feed and water intake and weight of rats in all the animals. However, number of faecal pellets and water in faecal pellets of the constipated rats changed significantly ($p < 0.05$) compared to the normal control. The administration of aqueous root extract of *Cida acuta* to the rats counteracted these alterations in a dose- dependent manner.

Table 2: Effect of Aqueous Extract of *Cida acuta* on Feed and Water Intake, Body Weight Gain and Faecal Properties of Constipated Rats after Treatment

Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Feed intake (g)	16.68±1.13	18.44±2.02	18.16±2.35	18.23±1.98	17.21±1.67	17.15±2.23
H ₂ O intake (ml)	36.33±3.12	33.54±2.41	35.45±2.81	36.37±2.33	35.12±2.81	35.29±2.49
No of faecal pellets	62.55±3.13 ^b	42.39±2.88 ^a	47.35±1.97 ^a	50.26±1.43 ^a	61.51±3.23 ^b	60.78±3.27 ^b
H ₂ O content of faecal pellets (ml)	3.48±0.05 ^b	1.24±0.56 ^a	2.11±0.15 ^a	3.16±0.77 ^b	3.41±0.87 ^b	3.05±0.66 ^b
Wt of rats (g)	3.54±0.12	3.05±0.44	3.71±0.32	3.44±0.45	3.59±0.22	3.68±0.36

Data are mean ± SEM values (n = 4); ^a significantly different (P < 0.05) compared to the normal control, ^b significantly different (P < 0.05) compared to constipated control. Group 1: Normal control and received 1ml distilled water. Group 2: Constipated

control and received 1ml distilled water. Group 3: Constipated and received 200 mg/ kg CAAE, Group 4: Constipated and received 400 mg/ kg CAAE, Group 5: Constipated and received 800 mg/ kg CAAE, Group 6: Constipated and received 0.21mg/ kg Bisacodyl

3.4 Effect of the Aqueous Root Extract of *Cida acuta* on Gastrointestinal Transit Ratio in loperamide-induced Constipated Rats

Figure 1 shows the effect of the aqueous root extract of *Cida acuta* on gastrointestinal transit ratio in loperamide-induced constipated rats. At all doses tested, the administration of the extract of *Cida acuta* to constipated rats significantly ($p < 0.05$) and dose- dependently increased gastrointestinal motility compared to the constipated control and it compares favorably well with bisacodyl, the standard drug used in this study.

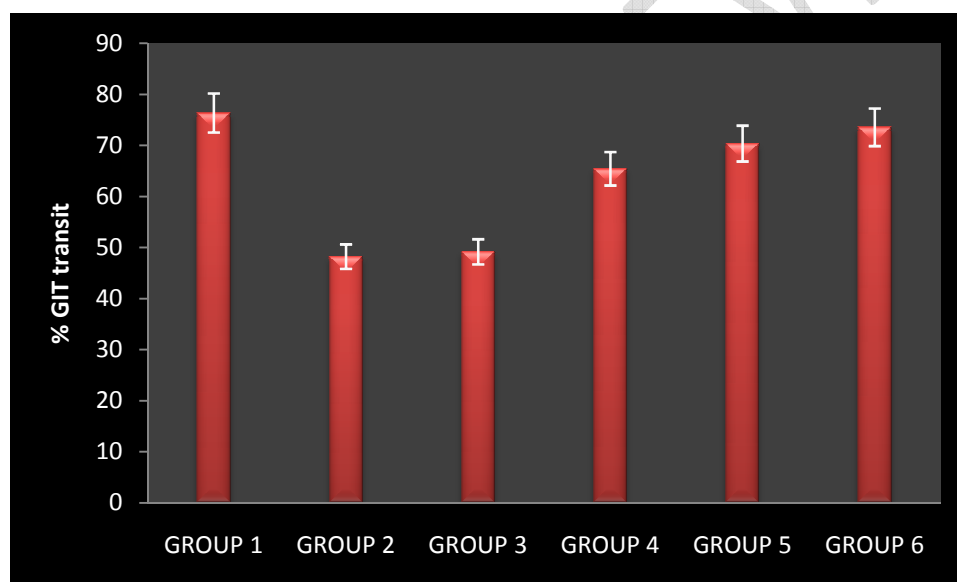


Figure 1: Effect of the aqueous root extract of *Cida acuta* on gastrointestinal transit ratio in loperamide-induced constipated rats.

4.0 DISCUSSION

The use of loperamide as a constipation inducer is well documented. The drug is an opioid agonist antidiarrheal that inhibits intestinal water secretion and colonic peristalsis^{[17], [18]}. This inhibition extends fecal evacuation time and delays intestinal luminal transit^[19]. Loperamide induced constipation is therefore considered to be a model of spastic constipation^[20].

The observed reduction in the number of faecal pellets and water content of faecal pellet following treatment with loperamide indicated induction of constipation in the rats. This observation was also reported in previous studies ^[21]. However, loperamide did not prevent the animals from feeding adequately ^[22]. The administration of the extract of *Cida acuta* to the constipated rats was effective in normalizing defecation frequency, faecal volume and motility of the colon. These are indications of the laxative property of the plant extract. This may be due to the presence of natural anthraquinone derivatives constituent of the leaf extract ^[23] which has been implicated to have laxative effects. This compound possibly exerts its action by disturbing the equilibrium between the absorption of water from the intestinal lumen via an active sodium transport and the secretion of water into the lumen by prostaglandin dependent mechanism ^[24].

The GIT ratio of the entire tract showed the overall gastrointestinal motor activity. This is very useful in constipation, abdominal bloating and refractory irritable bowel syndrome. It also provides quantitative information about colonic transit enables the identification and characterization of transit abnormalities and allows assessment of the severity of the disease as well as the response to treatment. ^[25]. The result presented herein clearly indicates that the *Cida acuta* leaf extract increased GIT motility in the constipated rats. This is consistent with the observation that was reported by Wintola *et al.*, ^[26] in a particular study. Furthermore, effect of the extract of *Cida acuta* leaves was interestingly dose dependent in this study. The results showed that the aqueous extract of *Cida acuta* leaves significantly increased the propulsion of carmine. The propulsion of carmine is probably due to the increasing of peristaltic movement in rat gastrointestinal tract resulting from the stimulation of cholinergic receptors by aqueous extract of *Cida acuta* leaves. The intestinal transit is controlled by both neural and myogenic mechanisms ^[27]. The observed laxative activity of *Cida acuta* lends credence to the fact that the plant may have high amount of water content. The Presence of phytoconstituents like terpenoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids ^[28] have been previously found to be responsible for laxative activities in plants.

5.0 Conclusion

Current research showed significant laxative effect of aqueous extract of *Cida acuta* in rats. Thus, this study provides sound scientific basis for the medicinal use of *Cida acuta* in constipation. Further research is required to assess the possible exact mechanism of the extract.

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