Original Research Article

ANTIDIARRHEAL ACTIVITY OF ETHANOL AND CHLOROFORM SEED EXTRACT OF *COLA NITIDA* IN EXPERIMENTALLY INDUCED DIARRHEA.

ABSTRACT

BACKGROUND: *Cola nitida* has been used in traditional medicine to treat diverse ailments including diarrhea. This study is carried out to investigate the antidiarrheal activity of the ethanol and chloroform seed extract of *Cola nitida* in Wistar albino rats.

METHOD: The ethanol and chloroform extracts of *Cola nitida* were evaluated with different doses (100mg/kg, 300mg/kg and 650mg/kg of animal weight) orally for antidiarrheal activity using castor oil induced-diarrhea, gastrointestinal motility test and castor oil-induced gastroenteropooling in Wistar albino rats. The observed activity was compared to standard antidiarrheal drug Lopermaide hydrochloride (2mg/kg) and to distilled water (10ml/kg) which served as the negative control.

RESULTS: *Cola nitida*ethanol extract at 150, 300 and 650mg/kg showed 55.64%, 59.73%, and 71.34% inhibition in gastrointestinal motility respectively. A significant reduction in diarrheal episodes ($p<0.0001$) was also observed with 650mg/kg of both extracts showing 100% inhibition. A reduction in the volume of fluid in the small intestine was also seen, this was however not significant. The chloroform extract of *Cola nitida* on the other hand produced a significant reduction in volume and weight of small intestinal content ($p<0.05$) with 650mg/kg showing a 92.73% inhibition of intestinal fluid accumulation.

CONCLUSION: The ethanoland chloroform extract of *Cola nitida*showed anti-diarrheal activity in animal model by decreasing the frequency of defecation and by reducing gastrointestinal motility and intraluminal fluid accumulation in the intestine.

Keywords: chloroform, *Cola nitida*, diarrhea, ethanol, rats

Introduction

Diarrheal disease is the second leading cause of death in children under five years old, and is accountable for the deaths of approximately 525 000 children every year [1]. As a result of this, The World Health Organization set in motion a control program in 1988 to investigate traditional medical practices and other associated areas [2]. Diarrhea can last several days, and can leave the body without necessary water and salts needed for survival. Formerly,the principal causes of deaths from diarrhea were severe dehydration and fluid loss. Now, other causes such as bacterial infections are likely to account for an increasing proportion of all diarrhea-related deaths. Diarrhea occurring in malnourished children and people living with HIV could be potentially fatal[3].

In Ghana it is the third leading cause of death in children under five years killing about 10,000 every year[4]. Diarrhea can have undesirable effects on the growth and cognitive development of children[5]. An estimated 94% of the diarrheal disease burden is attributable to the environment, and associated with risk factors such as unsafe drinking water, poor socio economic status, lack of proper sanitation and poor hygiene [6].

*Cola nitida*fruits have been employed traditionally as an aphrodisiac, appetite suppressant, to alleviate morning sickness, migraine, and indigestion [7].It has also been used to relieve inflamed or wounded skin [8]. The bitter twigsof *Cola nitida*have also been used for teeth and gum

cleaning[9]. *Cola nitida* is indigenous to West Africa and the nuts are obtained from cola trees. Cola has a broad number of species that have been widely cultivated some of which are*Cola anomala*, *Cola verticillata* (thonn.)Stapf, *Cola acuminate* (Pal.de Beauv) Schott and Endl. and *Cola nitida* (Vent) are the most prevalent of the edible species [10]. The fruits are commonly used by students, drivers, and other menial workers to prevent hunger and thirst and as stimulant to keep awake and combat exhaustion[11].

Cola trees are best known for their seeds or nuts which are rich in caffeine and other secondary metabolites such tannins phenols and xanthine[12]. The plant has also been reported to possess antidepressant and antidiarrheal activity [11]. Castor oil is known to induce diarrhea through its active metabolite, ricinoleic acid which stimulates peristalsis in the small intestine thus leading to changes in the electrolyte permeability of the intestinal mucosa[13].

Since cola nut is believed to possess antidiarrheal activity by traditionalists, this research is carried out to investigate the effect of the ethanol and chloroform crude extract of*Cola nitida* on diarrhea because very few literatures have been reported in this area. When confirmed pharmacologically, *Cola nitida* stands the chance of further studies to isolate the active constituent responsible for activity.

Materials and Methods

Plant Material

Cola nitida seeds were procurred from Akwatia, a town in the Eastern Region of Ghana. The samples were identified and authenticated by the Department of Pharmacognosy and Medicinal Chemistry School of Pharmacy, Central University, Ghana. A specimen of the samplewas submitted to the University's herbarium.

Plant Preparation and Extraction

The *Cola nitida*seeds were crushed into granules using mortar and pestle. A quantity of the comminuted granules equivalent to 2580.16grams was extracted with ethanol(70%) and 1500grams extracted with 1500milliteres of chloroform by cold maceration [14]. The mixture was shaken vigorously to enhance the extraction process and filtered after seven days to obtain the filtrate. The filtrate was evaporated using rotary evaporator (Drawell RE100 pro)to obtain the dry crude extract which was then stored in a refrigerator at 4^0C until ready for use.

Phytochemical Screening

The ethanol and chloroform crude extracts of *Cola nitida*were investigated for the presence of the following phytochemical constituents: tannins, saponins, phlobatannins, reducing sugars, alkaloids, flavonoids, cardiac glycosides using standard methods [14].

Experimental Animal

Wistar albino rats (95-120g) of both sexes obtained from The University of Ghana Animal House were used for the experiment. The animals were kept in standard plastic cages in a room with controlled 12hours light and dark cycle. They had unlimited access to clean water and were fed with standard pelleted commercial feed. The animals were allowed to acclimatize for 14 days prior to the experiments.The study was carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals[15] and Organization for Economic Cooperation and Development (OECD) guidelines [16]. The experiment was carried out in the Pharmacology Laboratory of Central University Ghana.

Acute Toxicity Study

The acute toxicity of *Cola nitida* was determined through the oral route. The animals were fasted for 24 hours and doses up to 2000mg/kg[16] of the ethanoland chloroform extract of *Cola nitida* were administered to rats of weight between the range of ninety to one hundred gram (90-100grams) orally and rats were observed closely for the first six hours and subsequently periodically for seven days for mortality and any delayed toxic manifestations.

Gastro-Intestinal Motility Test

Gastrointestinal (GI) motility test was carried out according to standard methods[17]with slight modifications. Transit time of gastrointestinal content was measured at three doses of the ethanol and chloroform*Cola nitida* extract (150mg/kg, 300mg/kg and 650mg/kg) with distilled water (10ml/kg) as negative control and Loperamide hydrochloride (2mg/kg) as positive control. All administrations were done orally with an oral gavage. All rats were administered 1ml of activated charcoal which served as a marker one hour after pretreatment. Rats were then sacrificed by cervical dislocation. The small intestines (from pylorus to caecum) were harvested and distance travelled by activated charcoal was measured and percentage inhibition of gastrointestinal motility was calculated.

Castor Oil-Induced Diarrhea Test

This experiment was carried out according to standard methods [1920]. Rats were fasted for 18hours and were divided into five groups. The nature of fecal matter (put into three categories solid, semi-solid, liquid), and frequency of defecations were measured over a period of six (6) hours. Rats in the first group received distilled water (10ml/kg), group two received standard drug Loperamide hydrochloride (2mg/kg)while groups three, four and five received 100mg/kg, 300mg/kg and 650mg/kg of ethanol and chloroform*Cola nitida* extracts respectively. Castor oil (1ml) was used to induce diarrhea in all experimental groups one hour after administration. Animals were placed in individual cages lined with absorbent paper. Percentage inhibition of diarrhea was calculated.

Castor Oil-Induced Gastroenteropooling Test

The effect of *Cola nitida* on the inhibition of intraluminal fluid accumulation was ascertained by measuring the volume and weight of fluid accumulated in the small intestine over a period of time[21]. Rats were divided into five groups of five rats and pretreated as described above. One hour after pretreatment, rats were administered 1ml of castor oil and were sacrificed after another hour by cervical dislocation. The small intestine from the pylorus to caecum was harvested and the contents of each small intestine was emptied into a graduated measuring cylinder and weighed. The volume and weight was recorded and percentage inhibition of secretion was calculated.

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 8.0. Results were summarized as mean ± SEM (n=5). Multiple comparison tests were determined by one-way ANOVA followed by post-hoc Tukey's honest significant difference (HSD) test. $P< 0.05$ was considered statistically significant.

Results

Phytochemical Screening

Results of different chemical tests on the ethanol and chloroform extracts of the seeds of *Cola nitida* showed the presence of tannins, saponins and other constituents.

Table 1: Results of phytochemical screening of ethanol and chloroform seed extract of *Cola nitida*

Phytochemical Constituent	*Cola nitida* (Ethanol extract)	*Cola nitida* (Chloroform extract)
Tannins	+	+
Saponins	+	+
Phlobatannins	+	+
Reducing Sugars	+	+
Alkaloids	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Phenols	+	-
Anthraquinones	+	+

Present (+); Absent (-)

Acute Toxicity Test

Oral administration of doses up to 2000mg/kg of the ethanol extract of *Cola nitida* did not produce any mortality nor any visible toxic manifestations. The chloroform extract of *Cola nitida* produced mortality at 2000mg/kg (50%), no deaths were observed when the dose was reduced to 1000mg/kg.

Gastrointestinal motility test

A significant dose-dependent inhibition of intestinal motility was observed by the ethanol ($p<0.01$ to $p<0.001$) and chloroform ($p<0.05$) extract of *Cola nitida* compared to the negative control as described in the table below. Loperamide hydrochloride produced the highest anti-gastrointestinal motility effect than the highest does of both the ethanol and chloroform extracts.

Table 2: Effect of ethanol and chloroform extracts of *Cola nitida* on inhibition of gastrointestinal motility

Treatment group	Dose (mg/kg)	Average length of small intestine/cm	Distance travelled by charcoal meal/cm	Percentage inhibition/%
Distilled water	10	74.58	62.10±4.46	16.73
Loperamide HCl	2	86.06	18.36±4.60****	78.67
Cola nitida (Ethanol Extract)	150	77.46	34.36±5.15**	55.64
	300	75.00	30.20±3.22***	59.73
	650	77.80	22.30±5.54****	71.34
Cola nitida (Chloroform Extract)	150	70.20	66.6±0.58*	5.13
	300	70.60	63.2±3.11*	10.48
	650	77.40	68.1±4.42*	12.02

Results are expressed as Mean±SEM (n=5). *P<0.05 **P<0.01, ***P<0.001, ****P<0.0001

Castor oil-induced diarrheal test

The ethanol($p<0.0001$) and chloroform ($p< 0.0001$) extracts of *Cola nitida*significantly inhibited diarrhea induced by the administration of castor oil with 650mg/kg of both extracts producing

100% inhibition of diarrhea. All doses of the ethanol and chloroform extract significantly reduced the frequency of watery stools.

Table 3: Effect of ethanol and chloroform extract of *Cola nitida* on castor oil-induced diarrhea in rats

Treatment groups	Dose (mg/kg)	Average no. of watery stools ± SEM	Percentage inhibition of diarrhea/%
Distilled water	10	12.00±0.4472	0.00
Loperamide HCl	2	1.000±0.3162****	91.67
Cola nitida (Ethanol Extract)	150	5.200±1.393****	56.67
	300	2.000±1.140****	83.33
	650	0.000±0.000****	100
Cola nitida (Chloroform Extract)	150	3.2±0.8602****	73.33
	300	2.4±1.122****	80.00
	650	0.000±0.000****	100

Results are expressed as mean ± SEM (n=5). ****P<0.0001

Castor oil-induced gastroenteropooling

The chloroform extract of *Cola nitida* caused a significant reduction in the volume (p=0.0039) and weight of small intestinal content (p<0.05) with 650mg/kg producing 92.73% reduction in volume of intestinal content. A reduction was also observed with the ethanol extract, this was however not significant.

Table 4: The effect of the ethanol and chloroform seeds extract of*Cola nitida* on castor oil-induced gastroenteropooling

Treatment groups	Dose (mg/kg)	Av. weight of small intestinal content ± SEM	Av. volume of small intestinal content ± SEM	Reduction in volume of intestinal content/%
Distilled water	10	1.354±0.16	1.10±0.02	0
Loperamide HCl	2	1.574±0.13	0.42±0.19	61.82
Cola nitida (Ethanol Extract)	150	1.148±0.36	0.44±0.19	60.00
	300	1.186±0.13	0.40±0.18	63.64
	650	1.574±0.35	0.60±0.28	45.45
Cola nitida (Chloroform Extract)	150	0.632±0.13**	0.46±0.09	58.18
	300	1.920±0.17*	0.58±0.15	47.27
	650	0.600±0.15**	0.08±0.08**	92.73

Results are expressed as mean±SEM (n=5). *P<0.05, **P<0.01 compared to the control

Discussion

Plant or plant parts are used traditionally for the management of diarrheal episodes without any scientific evidence to validate their use. This study was carried out to evaluate the antidiarrheal activity of the ethanol and chloroform extracts of *Cola nitida*, which are considered to be effective in the management of diarrhea among the Ashantis in Ghana. Castor oil-induced diarrhea test model, gastrointestinal motility test and castor oil-induced enteropooling test were employed to ascertain the anti-diarrheal activity of *Cola nitida* extracts in this study.

Castor oil-induced diarrhea test is employed to evaluate the anti-diarrheal activity of plants. Ricinoleic acid, which is the active constituent of castor oil is implicated in the its diarrheal effect by stimulating peristaltic activity in the small intestine leading to a change in permeability of electrolyte in the intestinal mucosa. It can also stimulate the release of endogenous prostaglandins which in turn result in the stimulation of secretion and motility [22]. Alkaloids, tannins, flavonoids and saponins are some of the phytochemical constituent present in the ethanol and chloroform extracts of *Cola nitida* (Table 1).

This study showed that ethanol and chloroform extracts of *Cola nitida* had antidiarrheal activity in all experimental models used. In the gastrointestinal motility test, the extracts decreased the transit of charcoal meal dose dependently (Table 2). The ethanol extract at 150mg/kg, 300mg/kg and 650mg/kg showed higher inhibition of gastrointestinal motility (55.64%, 59.73% and 71.34%) compared to the chloroform extract at the same doses (5.13%, 10.48% and 12.02%). The percentage inhibition of gastrointestinal motility was comparable to that shown by the standard drug Loperamide hydrochloride. A reduction in the motility of the muscles in the gastrointestinal tract lengthens the time substances spend in the intestine thereby allowing for more water absorption[23]. It can therefore be postulated that the reduction in gastrointestinal propulsion observed may be as a result of the anti-motility properties of the constituents present in the*Cola nitida* extracts. Studies have reported the anti-diarrheal activity of tannins and flavonoids as a result of their ability to reduce motility in the small intestine [2425].

In the castor oil-induced diarrhea test, the ethanol and chloroform extract of *Cola nitida* produced significant reduction in the number of watery stools which may be due to its ability to inhibit the synthesis of prostaglandin stimulated by the action of castor oil. Maximum anti-diarrheal effect was observed with the highest dose (650mg/kg) of the ethanol extract of *Cola nitida* (Table 3) rather than the standard antidiarrheal drug Loperamide hydrochloride. This might also be as a result of the phytochemical constituents like tanins, alkaloids and saponins present in the extract (Table 1) that may increase the time for water and electrolyte absorption by inhibiting intestinal motility[26].

For the castor oil-induced gastroenteropooling test, the chloroform extract of *Cola nitida* showed better activity compared to the ethanol extract (Table 4). The chloroform extract was able to significantly inhibit the accumulation of intraluminal fluid relative to the control and the maximum reduction in volume of small intestinal content was shown by the highest dose of the extract. It can therefore be postulated that the inhibition of intestinal fluid accumulation observed may be due to the inhibition of prostaglandin release and consequently increasing the reabsorption of water and electrolytes.

All doses of the ethanol extract of *Cola nitida* showed better anti-diarrheal activity in the gastrointestinal motility test and castor oil-induced diarrhea test than the chloroform extract which showed significant activity in the castor oil-induced gastroenteropooling test. This activity may be due to the phytochemical constituents in the extracts working singly or together.

Conclusion

This study showed thatthe ethanol and chloroform seed extract of*Cola nitida*extract possessed significant anti-diarrheal activity which may be due to the presence of phytochemical constituents like tannins, flavonoids, saponins and alkaloids. This study therefore provides pharmacological basis for the use of *Cola nitida*for the management of diarrhea in some rural communities in Ghana.

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