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ANTIDIARRHEAL STUDY OF METHANOL LEAF EXTRACT OF *ANTHOCLEISTA DJALONENSIS*

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ABSTRACT

The anti-diarrhoeal properties of methanol leaf extract of *Anthocleista djalonensis* on diarrhoea models (castor oil induced diarrhoea and castor oil induced fluid accumulation and intestinal transit models) were evaluated in rats. Five groups of five rats each were fasted for 18h and given 1ml/kg distilled water, 250, 500 and 1000mg/kg of the extract and 5mg/kg Loperamide respectively. The extracts caused a significant ($p<0.001$) decrease in the number of faecal matter passed and intestinal fluid accumulated in the castor oil-induced diarrhea and entreopooling models respectively, with 28.07 – 84.21% inhibition of defecation and 37.18 – 64.53% inhibition of intestinal fluid accummulation respectively in the two models. Furthermore, there was significant ($p<0.01$– $p<0.001$) decrease in intestinal transit in the castor oil-induced intestinal transit model with 28.71 - 68.51% inhibition of propulsive movement of the small intestine. From the foregoing, *Anthocleista djalonensis* leaf extract has been shown to inhibit the severity of diarrhoea, reduce water and electrolyte secretion into the small intestine and decrease intestinal transit. These findings lend credence to the use of the leaf of the plant in traditional medicine for the management of diarrhoea in some communities in Nigeria

KEYWORDS: Anthocleista Djalonensis, Diarrhoea, Castor Oil, Entreopooling, Intestinal Transit.

INTRODUCTION

Diarrhoeal diseases are the major causes of illness and death all over the world. Around 88% of diarrhoeal-related deaths are caused due to inadequate sanitation and poor hygiene [1]. Diarrhoeal diseases are one of the leading causes of childhood morbidity and mortality in developing countries. Diarrhoea causes an estimated 5 million death in children below 4 years of age per year.[2]

Several African medicinal plants have been reported to be useful in the treatment, management and/or control of diarrhea[3,4,5]. *Anthocleista djalonensis* (A. Chev.) is widely used for treatment of various ailments in Nigeria. The alcoholic leaf extract is taken to treat diarrhoea and dysentery. This study seeks to assess the anti-diarrhoeal properties of the methanol leaf extract of *Anthocleista djalonensis* using standard experimental protocols.

MATERIALS AND METHODS

Plant material and Preparation of plant extract

Anthocleista djalonensis leaves were collected from Alakahia in Port Harcourt, Nigeria. It was identified and authenticated at the University of Port Harcourt hebarium by a taxonomist. Pictorial and voucher samples were deposited at the herbarium with reference number, UPH/NO-P-053.

The leaves were shade-dried and subsequently grinded to coarse powder with an electric grinding machine. 2kg of the powder was macerated in 80% aqueous methanol for 72 hours, with intermittent shaking. At the end of three days, the mixture was poured through double layered muslin cloth to get rid of the debris and the fluid portion was filtered through Whatman No. 1 filter paper. The collected filtrate was concentrated to thick semi solid mass at 37^{o}C with a rotary evaporator (R210, Buchi, Switzerland) under reduced pressure and further freeze dried and kept in containers and stored in a refrigerator. Fresh stock solutions of the extract were made for the experiments.

Evaluation of antidiarrheal activity of the extract

Effect of extract on castor oil-induced diarrhea

25 rats were used for this study, they were randomly allocated to 5 groups of 5 rats per group. Prior to the commencement of the experiment, the rats were fasted for 18h, during which time the animals were given access to water *ad libitum*. The animals were pretreated as follows: Group 1 was treated with 10ml/kg (p.o.) of distilled water (the vehicle). Groups 2–4 received 1000, 500 and 250 mg/kg (p.o.) respectively of the extract. Group 5 was given 5 mg/kg (p.o.) loperamide, a standard antidiarrhoeal agent. Diarrhoea was induced in the rats

by administration of castor oil (3ml/kg, p.o.) thirty minutes after pretreatment with the extract and the standard drug, as described by.[5,6,1] Each rat belonging to a group was placed separately in a plastic cage lined at the bottom with a weighed clean sheet of paper. The animals were individually observed for 6h. The following parameters were recorded: (i) the time elapsed between the administration of castor oil and the excretion of the first diarrheic faeces, (ii) the total number of fecal output and the number of diarrheic stools excreted by each animal in 6 h.

Percentage inhibition of defecation was calculated by using the formula

Percentage inhibition =

$$\left[\frac{\text{Control Mean - Treated (Test) Mean}}{\text{Control Mean}} \times 100\right]$$

Effect of extract on castor oil-induced intestinal transit: 25 rats were fasted for 18 h but the animals were given access to water *ad libitum.* They were randomly allocated into five groups of 5 rats each. The rat in each group was separately placed in a plastic cage lined at the bottom with a transparent sheet. Group1 served as control and was given 10 ml/kg (p.o.) of distilled water. Groups 2 - 4 received 1000, 500, and 250 mg/kg (p.o.) respectively of the extract. Group 5 was treated with 5 mg/kg (p.o.) of loperamide. These treatments were all followed by oral administration of 3ml/kg of castor oil after thirty minutes. After a further thirty minutes following oral treatments of the animals with castor oil, each animal was given 3ml/kg (p.o.) of 10% activated charcoal in normal saline. Forty minutes after the charcoal meal, the rats were anaesthetized by ether inhalation and euthanized by cervical dislocation. The abdomen of each rat was cut open, and the whole length of the intestine from the pylorus to the caecum was ligated, dissected and carefully removed. The distance of the charcoal plug from pylorus to caecum, i.e., peristaltic index (PI) which is the distance traveled by charcoal meal relative to the entire length of the small intestine was determined and expressed as a percentage of the total length of the small intestine according to the method described by Dicarlo *et al* [7]. Percentage inhibitions of intestinal transit of the charcoal meal was using the formula:

Percentage inhibition =

$$\left[To - \frac{Tt}{To}\right] \times 100$$

where

To = mean length traversed by charcoal meal in distilled water-treated control rats;

Tt = mean length traversed by charcoal meal in extract and loperamide-treated test rats.

Effect of extract on Castor oil-induced enteropooling, and intestinal fluid

5 groups of 5 rats per group were fasted for 18 h but with free access to drinking water. The rats were pretreated as follows: Group1 was given10 ml/kg (p.o.) distilled water, Groups 2 - 4 received 1000, 500 and 250 mg/kg (p.o.) of extract respectively, Group 5 was treated with 5mg/kg (p.o.) loperamide. Thirty minutes after pretreatment, each rat in the five groups was given 3 ml/kg (p.o.) castor oil. Every rat in each group was separately placed in a plastic cage lined at the bottom with a transparent sheet.

The volume of intestinal fluid in the rats were then measured as described in detail by Dicarlo *et al*, [7] with some modifications. Forty minutes after castor oil was given, the rats were anaesthetized by ether inhalation and euthanized by cervical dislocation. The abdomen of each rat was cut open, and the whole length of the intestine from the pylorus to the caecum was ligated and carefully removed. The weight of the full intestine was determined. The intestinal content was expelled into a graduated measuring cylinder and its volume was determined. The weight of the empty intestine was taken, and the difference between the full and empty intestine was thus calculated.

The Effect of Leaf Extract On Castor OIL-Induced Diarrhoea

Treatment Dose (mg/kg)	Onset of Diarrhoea (mins)	Mean wet faeces % Inhibition
Control	36.00 ±11.75	11.40 ±0.54
250	65.00 ±9.63	8.20 ±0.66** 28.07
500	86.40 ±25.08	3.00 ±0.63**** 73.68
1000	98.00 ±28.14	1.80 ±0.86**** 84.21
Loperamide.	0.00 ± 0.00	0.00± 0.00**** 100

** = $p<0.01$ **** = $p<0.0001$ n=5

4.1.6 Effect of Extract On Castrol Oil- Induced Enteropooling.

Dose mg/kg	Wt. of intestine with fluid	Wt. of intestine alone	Vol. of intestinal fluid (ml).	% Inhibition
Control	9.18±0.41	4.31±0.16	4.68±0.32	
250	10.21±0.79	7.31±0.51	2.94±0.22***	37.18
500	8.84±0.65	6.41±0.62	2.02±0.12****	56.84
1000	8.84±0.87	6.83±1.05	1.66±0.31****	64.53
Loperamide	8.79±1.50	6.92±1.20	0.86±0.07****	81.62

*** = p<0.001 **** = p<0.0001 n=5

The Effect of LEAF Extract on Castor Oil Induced Intestinal Transit.

Treatment Dose (mg/kg)	Full length of intestine(cm)	Length travelled by charcoal(cm)	% Inhibition
Control	111.20 ± 4.97	90.20± 5.24	
250	126.80± 10.55	64.30± 4.99**	28.71
500	111.00 ± 2.74	47.20 ± 4.16****	47.67
1000	101.80± 1.02	28.40 ± 3.49****	68.51
Loperamide	122.60± 7.20	15.20± 3.55****	83.14

** = p<0.01 **** = p<0.0001 n=5

DISCUSSION

Diarrhoea is evoked by hyperpropulsive motility of gastrointestinal tract and hypersecretion throughout the intestinal mucosa. Castor oil is often used to induce diarrhoea. It contains ricinoleic acid, which causes changes in electrolytes and water transport and generates enormous contractions in the transverse and distal colon[8] thereby producing permeability changes in the intestinal mucosal membranes that result in watery luminal content that flows rapidly through the small and large intestines.[9,10] Ricinoleic acid from castor oil causes irritation and inflammation of the intestinal mucosa, due to its tendency to cause the colonic cells to release prostaglandins and other mediators of inflammation, these mediators stimulate motility and secretion.[11,12]

In castor oil-induced diarrhoeic animals, Anthocleista djalonensis leaf extract dose dependently and significantly delayed the onset of copious diarrhoea, reduced the number of wet stools and inhibited the severity of diarrhoea. Since the extract was capable of inhibiting the castor oil induced diarrhea, we can suppose that inhibition of the synthesis of prostaglandins and other mediators of inflammation may be involved in the mechanism of its action.

In the enteropooling study, the extract significantly reduced the volume of the animals' intestinal fluid content. The intraluminal fluid accumulation induced by castor oil was blocked by the extract in a dose-related manner. Clinically, diarrhea may result from disturbed bowel function, in which case, there is impaired intestinal absorption, excessive intestinal secretion of water and electrolytes, and a rapid bowel transit.[13,5]

The significant inhibition of the castor oil-induced enteropooling by Anthocleista djalonensis leaf extract in rats suggests that the extracts probably produces relief in diarrhoea through its anti-enteropooling effects. These effects, which are direct consequences of reduced water and electrolytes secretion into the small intestine, suggest that the extracts may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hypersecretion earlier indicated. However, since electrolyte absorption determines the efficiency of nutrient absorption[14], it is likely that the enhanced electrolyte absorption by the extract may have encouraged the absorption of other intestinal contents. Also, solute absorption in any region of the intestine is a function of the rate of water uptake in that region.[15] Thus, the extract-enhanced solute absorption may have created an osmotic gradient across enterocytes which stimulated water absorption. These observations reasonably suggest that the extract inhibits gastrointestinal hyper-secretion and enteropooling by enhancing electrolytes, solutes and water absorption from the intestinal lumen.

The extract significantly inhibited small intestinal propulsive movement in rat. Inhibition by the highest dose was however lower than that of the standard drug, loperamide. Like the other opioids, loperamide's stimulation of the mu-opiate receptors in the periphery results in decreased peristalsis.[16,17,18,19] Furthermore, a previous study showed that calcium antagonism is responsible, at least in part, for the antidiarrheal effect of loperamide [20]. Earlier study of an alcoholic leaf extract of *Anthocleista djalonensis* showed in-vitro antispasmodic and smooth muscle relaxant activities, the effect of which is thought to be due to inhibition of calcium mobilization.[21]

It is therefore, not unreasonable to suggest that the calcium antagonist property of Anthocleista djalonensis leaf extract may be due at least in part to its antidiarrhoeal effect observed in this study.

Although the exact mechanism of the antidiarrheal action of Anthocleista djalonensis could not be established in this study, *Anthocleista djalonensis* is known to contain saponins and alkaloids[22], it could be said that the observed antidiarrhoeal effect of the plant extract stems

from the presence of these secondary metabolites because previous studies has shown that compounds such as flavonoids, triterpenoids, saponins and a host of other plant secondary metabolites possess antidiarrheal properties in various experimental animal models.[23,3,24,25,26]

CONCLUSION

The study has shown that *Anthocleista djalonensis* inhibited the severity of diarrhoea, reduced water and electrolytes secretion into the small intestine and decreased intestinal transit. All these translate to an anti-diarrheal effect. These findings therefore confirm the ethnomedical basis for the use of *Anthocleista djalonensis* in the management of diarrhea in some rural communities of Nigeria.

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