

Full Length Research Paper

Evaluation of antidiarrhoeal activity of the stem bark of *Cylicodiscus gabunensis* (mimosaceae)

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Accepted 28 March, 2006

The ethyl acetate (EA) extract of the stem bark of *Cylicodiscus gabunensis* (CG) (Mimosaceae) was analysed for its anti-diarrhoeal activity. Like loperamide (3 mg/Kg body weight), a single oral dose of *C. gabunensis* ethyl acetate extract (CG-EA) (375, 750 mg/Kg body weight) produced a significant decrease in the severity of diarrhoea. To understand the mechanism of its anti-diarrhoeal activity, its effect was further evaluated on intestinal transit, castor oil induced intestinal fluid accumulation (enteropooling) and electrolyte concentration in the small intestinal fluid. CG-EA produced a decrease in intestinal transit (10.26-30.75%), and unlike atropine, it significantly inhibited castor oil induced enteropooling. However, it did not alter the electrolyte concentration in intestinal fluid as compared to castor oil-treated rats.

Key words: *Cylicodiscus gabunensis*, antidiarrhoeal activity, castor oil.

INTRODUCTION

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases including diarrhoea. Diarrhoea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the paediatrics ward die of diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries (Fauci et al., 1998). According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhoea (Park et al., 2000). The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organisations to curb it. It is therefore important to identify and evaluate available nat-

ural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always free from adverse effects (Harman et al., 1992). A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these anti-diarrhoeal traditional medicines has not been scientifically evaluated.

Cylicodiscus gabunensis (Mimosaceae) commonly called denya (Ghana), edum (Gabon), adoum, Bokoka (Cameroon), bouemon (Ivory Coast) (Chudnoff et al., 1984) is a large tree with a cylindrical trunk. The stem is more or less pyramidal in shape. The bark has a strong odour with widespread branches. The leaves are imparipinnate sub sessile, alternate and are slightly asymmetrical. The inflorescence is on the branches. The flowers are small, 2 - 5 mm long and 2 - 3 mm wide. The pods are long, hanging up to 1 m long and 4 cm broad, acute at the base and acuminate at the apex (Adjanooun et al., 1996). *C. gabunensis* (CG) is found in The dense, humid forest and it is widespread from

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Cameroon to Cabinda (Adjanohoun et al., 1996). Four saponins were isolated from the alcoholic extract of the bark of CG (Tchivounda et al., 1991). Medicinally, the barks of the stem are used to prepare remedies for gastro-intestinal disorders, headache and rheumatism (Adjanohoun et al., 1996).

The present study investigates the anti-diarrhoeal activity of CG against experimentally induced diarrhoea.

MATERIALS AND METHODS

Treatment of animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of the Centre for Research in Food and Nutrition of the Institute of Medical Research and Medicinal Plant Studies (IMPM), Yaoundé, Cameroon. The rats were given food and water *ad libitum*. All the animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments. All studies were carried out in groups of 6 rats each. Each rat was housed separately in a metabolic cage.

Collection of plant materials

The barks of the stem of CG were collected in the morning on Mount Eloumdem, Yaoundé, Cameroon in January 2004. Identification of the plant was confirmed in the National Herbarium Yaoundé (reference number of the plant: 21574/SRF/Cam). The barks were then air-dried at room temperature. The dry barks were ground into a fine powder.

Preparation of the plant extract

This was done by soaking the dry plant powder (200 g) in a bottle (1.5 l) of ethyl acetate (EA) and kept for 72 h. The plant-ethyl acetate mixture was then sieved. The filtrate (extract) was concentrated by evaporating the ethyl acetate using a rotary evaporator. The *C. gabunensis* ethyl acetate extract (CG-EA) was further concentrated by allowing it to stand overnight in an oven at 30°C.

Drugs and chemicals

Atropine sulphate and loperamide (standard reference anti-diarrhoeal drugs), castor oil (laxative agent), normal saline solution (9% NaCl), charcoal meal (10% activated charcoal in 5% gum acacia) and vehicle (0.5% v/v Tweens 80 in distilled water) were used.

Castor oil-induced diarrhoea

24 rats were allowed to fast for 18 h and divided into 4 groups of 6 animals each. All groups received castor oil at a dose of 1 ml/animal orally (p.o.) (Doherty et al., 1981). 30 min after castor oil administration, the first group (control group) received vehicle (0.5% Tween 80 in distilled water), the second and third groups CG-EA 375 mg/kg and 750 mg/kg body weight, respectively. The fourth group received the reference drug, loperamide (3 mg/kg orally). After this administration, the animals were placed separately in metabolic cages with filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour for 6 h. The total number of faeces and diarrhoea faeces excreted and the total

weight of faeces were recorded within a period of 24 h and compared with the control group. The total number of diarrhoea faeces of the control group was considered 100%. The results were expressed as a percentage of inhibition of diarrhoea (Zaval et al., 1988).

Study of small intestinal transit

This was done according to the method proposed by Mujumdar et al. (1998) using charcoal meal as a diet marker. The rats were divided into 4 groups of 6 animals each. The first group (the control group) was orally administered the vehicle (0.5% Tween 80 in distilled water). The second and third groups orally received CG-EA, 375 mg/kg and 750 mg/kg body weight respectively. The fourth group also orally received the standard drug, atropine sulphate (5 mg/kg body weight). 30 min after administration, each animal was given 1 ml of charcoal meal orally (10% activated charcoal in 5% gum acacia). Also, 30 min after this administration, each animal was sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as a percentage of distance moved.

Castor oil-induced enteropooling and electrolyte secretion

Intraluminal fluid accumulation was determined by the method of Robert et al. (1976). The rats were divided into 5 groups of 6 animals each. Group one received 2 ml of normal saline solution and group 2 received 2 ml of castor oil. Groups 3, 4 and 5 received atropine sulphate (0.1 mg/kg) through the intraperitoneal route (i.p.), 375 mg/kg p.o. CG-EA and 750 mg/kg p.o. CG-EA, respectively, one hour before the oral administration of castor oil. Two hours later, the rats were anaesthetised with ether and sacrificed. The edges of the small intestine were tied with thread and the intestine was removed and weighed. The intestinal content was collected by squeezing it into a graduated tube and the volume measured. The intestine was reweighed and the difference between full and empty intestines was calculated. The Na⁺ and K⁺ concentrations were measured in the supernatant after centrifuging the intraluminal fluid by flame photometry.

Phytochemical screening

The freshly prepared extract was qualitatively tested for the presence of chemical constituents such as tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars. They were identified by characteristic colour changes using standard procedures (Odebody and Sofowora, 1978).

Statistical analysis

The values were expressed as mean ± standard deviation. The statistical analysis of data was by Analysis of Variance (ANOVA) using 5% level of significance. The statistical package used was SPSS 9.0. A One-way ANOVA enabled the significant differences between the values to be observed. The Duncan test was used to identify these differences.

RESULTS

Effect of CG-EA and castor oil-induced diarrhoea

In the castor oil-induced diarrhoea experiment, the rats that did not receive the plant extract, showed typical diarrhoea signs such as watery and frequent

Table 1. Effect of *C. gabunensis* ethyl acetate extract (CG-EA) on castor oil-induced diarrhoea.

Group	Total number of faeces	Total number of diarrhoeal faeces	Percentage of inhibition	Total weight of faeces (g)	Percentage of inhibition
Castor oil (1 mL) + vehicle (0.5% Tween 80)	24.83 ± 4.53 ^d	20.83 ± 5.115 ^b	0%	7.38 ± 2.20 ^c	0%
Loperamide (3 mg/kg) + castor oil (1 mL)	9.83 ± 0.93 ^a	6.17 ± 1.60 ^a	70.38%	1.76 ± 0.83 ^a	76.83%
CG-EA (375 mg/kg) + castor oil (1 mL)	21.50 ± 2.43 ^{cd}	4.33 ± 2.65 ^a	79.22%	5.04 ± 1.47 ^b	31.69%
CG-EA (750 mg/kg) + castor oil (1 mL)	20.50 ± 4.37 ^c	7.17 ± 1.32 ^a	65.58%	5.04 ± 0.58 ^b	31.71%

Values in the same column with different letter superscripts are significantly different ($p < 0.05$).

Table 2. Effect of *C. gabunensis* ethyl acetate extract (CG-EA) on charcoal-induced gut transit changes.

Group	Percentage of distance travelled by charcoal meal	Percentage of inhibition
Vehicle (0.5% Tween 80)	74.57 ± 9.90 ^d	0%
Atropine sulphate (5 mg/kg)	40.32 ± 4.25 ^a	45.93%
CG-EA (375 mg/kg)	66.93 ± 7.18 ^{cd}	10.26%
CG-EA (750 mg/kg)	51.64 ± 4.25 ^b	30.75%

Values in the same column with different letter superscripts are significantly different ($p < 0.05$).

defecation. The EA extract of CG produced a marked anti-diarrhoeal effect in the rats. Both doses of CG-EA significantly decreased ($p < 0.05$) the total number of wet faeces produced by administration of castor oil (4.33 at the dose of 375 mg/kg and 7.17 at the dose of 750 mg/kg) as compared to the castor oil-treated control group (20.83). The percentage of inhibition of castor oil induced diarrhoea in CG-EA treated rats was 79.22 and 65.58% respectively at 375 and 750 mg/kg dose.

The effect of CG-EA was similar to that of the standard drug, loperamide (3 mg/kg) which produced an inhibition of 70.38% (Table 1). The average weight of faeces in the control group was 7.38 g. Treatment with both doses of CG-EA significantly reduced ($p < 0.05$) the weight of faeces to 5.04 g (Table 1).

Effect of CG-EA on charcoal-induced gut in transit changes

The administration of CG-EA also slowed down the propulsion of charcoal meal through the gastro-intestinal tract when compared to the castor oil-treated rats. The percentage of intestinal length travelled by charcoal meal in CG-EA pre-treated (375 and 750 mg/kg) and castor oil-treated rats was 66.93, 51.64 and 74.58%, respectively. Atropine on its part, produced a marked decrease in the

propulsive movement and the intestinal length travelled by charcoal meal was 40.33 (Table 2).

Effect of CG-EA on castor oil-induced enteropooling and electrolyte secretion

CG-EA was also found to possess an anti-enteropooling activity. Oral administration of castor oil produced a significant increase ($p < 0.05$) in the intestinal fluid (3.12 ml) as compared to the normal rats (1.28 ml). CG-EA when given orally one hour before castor oil, significantly inhibited ($p < 0.05$) the enteropooling; 1.46 ml (375 mg/kg) and 1.66 ml (750 mg/kg). The volume of intestinal fluid was similar to that obtained in the normal group (1.28 ml) (Table 3). The weight of the intestinal content was also significantly decreased following treatment with castor oil (from 3.54 to 2.02 g in the normal rats). However, CG-EA produced a marginal decrease in the weight of the intestinal content. The secretions were more viscous. Treatment of rats with castor oil significantly increased ($p < 0.05$) the Na^+ concentration to 11 meq/l as compared to the control group (8.75 meq/l). CG-EA at both doses as well as atropine sulphate pre-treatment did not alter the Na^+ concentration in the intestinal fluid, similar to the castor oil treated group. None of the treatment produced a significant change in the K^+ concentration.

Table 3. Effect of *C. gabunensis* ethyl acetate extract (CG-EA) on castor oil enteropooling and electrolyte secretion.

Group	Volume of intestinal content (ml)	Weight of intestinal content (g)	Concentration of Na ⁺ (meq/l)	Concentration of K ⁺ (meq/l)
Castor oil (2 ml)	3.12 ± 0.37 ^d	3.54 ± 0.61 ^c	11.00 ± 1.50 ^a	5.64 ± 1.41 ^a
Normal saline (2 ml)	1.28 ± 0.24 ^{ab}	1.02 ± 0.25 ^{ab}	8.75 ± 1.49 ^{ab}	6.34 ± 0.98 ^{ab}
Atropine sulphate + castor oil (2 ml)	1.80 ± 0.31 ^c	2.46 ± 0.36 ^b	15.83 ± 3.46 ^{ab}	7.78 ± 0.20 ^{ab}
CG-EA (350 mg/kg) + castor oil (2 ml)	1.46 ± 0.28 ^{abc}	2.44 ± 0.24 ^b	15.68 ± 4.06 ^{ab}	7.39 ± 2.27 ^{ab}
CG-EA (750 mg/kg) + castor oil (2 ml)	1.66 ± 0.37 ^{bc}	2.40 ± 0.31 ^b	17.67 ± 7.27 ^b	7.79 ± 2.01 ^{ab}

Values in the same column with different letter superscripts are significantly different ($p < 0.05$).

DISCUSSION

Castor oil causes diarrhoea due to its active metabolite, ricinolic acid (Ammon, 1974; Watson, 1962), which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin (Galvez et al., 1993). In this study, the ethyl acetate extract of CG exhibited a significant anti-diarrhoeal activity. Its effect did not depend on the dose. The results were similar to that of the standard drug loperamide (3 mg/kg) with regard to the severity of diarrhoea. CG-EA significantly reduced intestinal transit as observed by the decrease in intestinal motility of charcoal meal. The extract also led to a marked reduction in the weight and the volume of the intestinal contents.

Phytochemical screening revealed the presence of tannins, sterol and/or triterpenes and reducing sugars (Mabeku et al., unpublished data). Earlier studies showed that anti-dysenteric and anti-diarrhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes and reducing sugars (Anonymous, 1962; Galvez et al., 1991, 1993; Longanga et al., 2000). Hence, tannins, reducing sugars, sterol and/or triterpenes may be responsible for the mechanism of action of CG-EA anti-diarrhoeal activity. The anti-diarrhoeal activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistant and hence, reduce secretion (Tripathi, 1994). This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal.

Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any effect on colonic motility (Theoderau et al., 1991). Atropine significantly reduced intestinal transit time. This is possible due to its anticholinergic effect (Brown and Taylor, 1996). However, it did not inhibit castor oil-induced enteropooling, thereby, suggesting that

mediators other than acetylcholine are involved in castor oil-induced enteropooling. Furthermore, a decrease in intestinal transit time with atropine could also be due to reduction in gastric emptying (Izzo et al., 1999).

In conclusion, the results of this investigation revealed that CG-EA contains pharmacologically active substance(s) with anti-diarrhoeal properties. These properties confirm the use of CG as an anti-diarrhoeal drug as proposed by traditional healers. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecules responsible for the anti-diarrhoeal activity observed.

ACKNOWLEDGEMENTS

We are grateful to the Centre for Research in Food and Nutrition (CRAN) of the Institute of Medical Research and Medicinal Plant Studies (IMPM) Yaoundé, for their material support. We are also grateful to Mrs ACHU Mercy Bih for reading through the manuscripts and making useful suggestions.

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