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Research Article

Phytochemical Screening and Antidiarrhoeal Activity of a Crude Extract of *Alchornea Cordifolia (Euphorbiaceae)* Leaves in Laboratory Rats

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Abstract

The treatment of common diseases remains a problem for African populations, despite the fact that African pharmacopoeia abounds with numerous medicinal plants, like Alchornea cordifolia, a plant used by many traditional healers as an anti-diarrhoeal. The aim of this study was to evaluate the in vivo anti-diarrhoeal potential of a decoction of Alchornea cordifolia leaves in experimental rats. Antidiarrhoeal activity was assessed in an experimental castor oil-induced diarrhoea model. It was evaluated in preventive and then curative model. Percentages of diarrhoeal stool inhibition were calculated in each case. Phytochemical screening was carried out in search for major phytochemical groups that could provide anti-diarrhoeal activity. In rats pre-treated with A. cordifolia leaf decoction, there was a reduction in the number of diarrhoeal episodes at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg bw. The percentages of inhibition were 34.21%, 76.80% and 52.83% respectively, compared to 54.10% for loperamide. A. cordifolia at doses of 200mg/kg to 400mg/kg also delayed the onset of diarrhoea. For the evaluation of curative antidiarrhoeal activity, administration of A. cordifolia decoctate led to a reduction in diarrhoeal episodes at doses of 100mg/kg, 200mg/kg and 400mg/kg bw. The percentages of inhibition were 39.35%, 63.59% and 50.16%. Loperamide inhibition was at a rate of 57.27%. Phytochemical screening revealed the presence of tannins known to be responsible for this anti-diarrhoeal activity. Alchornea cordifolia therefore has anti-diarrhoeal properties that could be used in phytomedicines.

Introduction

Diarrhoea is the first leading cause of death in children under five years old worldwide, particularly in developing countries. It is the cause of 525,000 child deaths per year specifically in developing countries [1]. In Côte d'Ivoire, it is the third leading cause of child mortality after malaria and pneumonia [2]. Dehydration due to acute diarrhoea is the main cause of death in children [3]. The administration of oral rehydration salts and zinc reduces mortality from diarrhoea [4]. However, the African region has difficulties ensuring equal access to healthcare, while the African pharmacopoeia is full of medicinal plants [5], like Alchornea cordifolia, a plant used by many traditional healers as an anti-diarrhoeal [6]. The aim of this study was therefore to evaluate the in vivo anti-diarrhoeal potential of Alchornea cordifolia leaf decoction in experimental rats.

Materials and Methods

Plant material

The plant material was made of Alchornea cordifolia (Schum. And Thonn.) leaves collected in February 2021 in the city of Korhogo (northern Côte d'Ivoire). The leaves were identified at the National Centre of Floristics of Abidjan, which is affiliated to the University of Félix Houphouët Boigny (Abidjan). A plant sample (AC 2021) was kept at the laboratory of Pharmacology. The leaves were dried in the laboratory at 18°C. The dried leaves were ground to obtain a fine powder.

Extraction method

To prepare the decoction, 100g of Alchornea cordifolia leaf powder was boiled in one litre of distilled water for 15 minutes. After cooling, the decoction was filtered four times on hydrophylic cotton. The filtrate obtained was put in an oven at 50°C for 72 hours. The dry residue obtained was scraped with a scalpel blade and ground in a porcelain mortar to get a dry decoction of A. cordifolia leaf. The extraction yield as well as the residual moisture were calculated. This decoctate was used to prepare test solutions of 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml based on previous studies corresponding to respective doses of 50 mg/kg, 100 mg/ kg, 200 mg/ kg and 400 mg/ kg [7].

Experimental animals

Wistar albino rats (Rattus norvegicus) weighing between 120g and 200g were obtained from the animal house of the laboratory of Pharmacology Laboratory, faculty of biology and pharmaceutical sciences, University of Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire). Animals were kept under standard conditions room of 24 ± 1°C with a 12 h of light 12 h and dark cycles. Animals had free access to water and food. Prior to experiment, they were kept into fasting for 12 hours and water was provided ad libitum.

Technical equipment

The following technical equipment for this tes were used: metabolic cage, scales, syringes, oral gavage feeding steel tube, blotting paper, watch glass, mortar and pestle, spatulas, beakers.

Solvent and reagents

For this study, castor oil (Dermopharm®) as an experimental diarrhoea-inducing agent, Loperamide 2mg capsule (Imodium®) as the reference antidiarrhoeal, and physiological saline were used.

Phytochemical screening

The phytochemical screening was done using the method described in the works of Békro et al. (2007) [8], Ronchetti and Russo (1971) [9] and Wagner (1983) [10]. These were saponosides, polyphenols (flavonoids and tannins), polyterpenes, quinonic substances and alkaloids.

Anti-diarrhoeal activity

Anti-diarrhoeal activity was assessed in an experimental model of castor oil-induced diarrhoea.

Preventive activity

Principle

Preventive administration of an antidiarrhoeal substance reduces the time of onset of diarrhoeal stools and the number of diarrhoeal episodes induced by castor oil in experimental animals [11].

Procedure

Six homogeneous groups of 6 rats per group were kept fasting for 6 hours. Group 1 (negative control) received normal saline by oral route. Group 2 (control) was given loperamide at a dose of 2mg/kg (standard drug) by gavage. Groups 3, 4, 5 and 6 were administered A. cordifolia leaf decoctate by oral route at doses of 50mg/g; 100mg/kg; 200mg/kg; 400mg/kg respectively. One hour (1h) later, each rat received 2ml of castor oil by gavage. The animals were then put individually in metabolic cages containing an absorbent paper to collect faeces and note the time of appearance of first diarrhoeal stools, then observed and noted for a period of 6 hours the duration of diarrhoeal episodes and the weight of diarrhoeal stools. The percentage of diarrhoea inhibition was calculated using the following formula:

$$I\,(\%) = \frac{(M - m)}{M} 100$$

I (%): percentage of diarrhoea inhibition
M: mean weight of diarrhoea stools in the control group (mg)
m: mean weight of diarrhoea stools in treated group (mg)

Curative activity

Principle

Administration of an anti-diarrhoeal substance to experimental animals in which experimental diarrhoea were induced by castor oil reduces the number of diarrhoeal episodes and the weight of diarrhoeal stools [11].

Procedure

Six homogeneous groups of 6 rats per group were kept fasting for 6 hours. Each rat in the different groups was given 2 ml of castor oil by oral route. As soon as the first soft faeces was released, substances were given to rats. Group 1 (control) received normal saline; group 2 received loperamide (standard drug) at 2mg/kg. Groups 3, 4, 5 and 6 received A. cordifolia leaf decoctate at 50mg/g; 100mg/kg; 200mg/kg; 400mg/kg respectively. The animals were then put individually in metabolic cages containing absorbent paper to collect faeces and observed and noted for a period of 6 hours, the duration of diarrhoeal episodes and the weight of diarrhoeal faeces. The percentage of diarrhoea reduction was calculated using the following formula:

$$R\,(\%) = \frac{(M - m)}{M} 100$$

R (%): percentage of diarrhoea reduction
m: mean weight of faeces from the treated group (mg)
M: mean weight of faeces from the control group (mg)

Data processing

Results were expressed as mean ± standard deviation. Graphs were produced using GraphPad Prism 8.0.2 software. The Wilcoxon test was used for statistical analysis. The difference between mean values was considered significant if $p < 0.05$.

Results

Extraction yield and residual moisture

Extraction yield was 13.98g/100g or 13.98%. The residual moisture was 8.75%.

Phytochemical screening

The decoction of A. cordifolia leaves contained polyphenols including flavonoids and catechic tannins, sterols, polyterpenes, alkaloids and saponosides.

Anti-diarrhoeal activity

Preventive anti-diarrhoeal activity

The time of onset of diarrhoeal stool was 3 hours for rats in the control group and 4 hours for rats in the standard drug group. With A.cordifolia leaf decoctate, the time of onset of diarrhoeal stools was 3h for doses of 50 and 100 mg/kg and 4h at doses of 200 and 400 mg/kg. The duration of diarrhoeal episodes was 5.66 ± 0.74 h for rats in the control group and 3.33 ± 0.47 h for rats in the standard drug group. For A. cordifolia leaf decoctate, the duration was 5.66 ± 0.47 h, 4.16 ± 0.70, 2.66 ± 0.47 and 3.83 ± 0.70 respectively for doses of 50, 100, 200 and 400 mg/kg. The mean weight of diarrhoeal faeces was calculated and shown in Figure 1: 2277.83 ± 710.07 mg for the control group and 1074.17 ± 734.50 mg ($p = 0.01$) for the standard drug group. For rats given A.cordifolia leaf decoctate the mean weight were 2164.50±1445.35 mg; 1498.67±662.69 mg; 538±884.65 mg ($p = 0.003$) and 1074.50±734.50 mg. The respective percentages of diarrhoea inhibition (I) were 5%; 34.21%; 76.38%; 52.83% compared to control group. That of loperamide was 54.10%. At a dose of 200mg/kg a significant reduction in the weight of diarrhoea stools was observed. The other doses also showed a reduction of weight of diarrhoeal faeces, but this reduction was not statistically significant **(Figure 1)**.

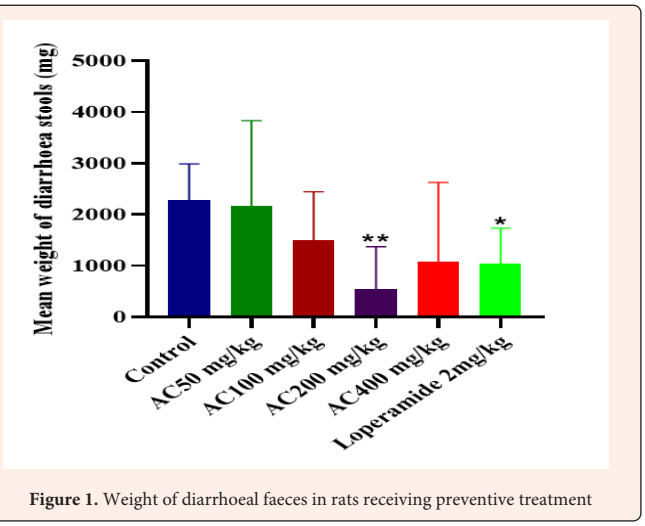

Figure 1. Weight of diarrhoeal faeces in rats receiving preventive treatment

Wilcoxon test: *: $p<0.05$ compared with control; **: $p<0.01$ compared with control.

Curative anti-diarrhoeal activity

Administration of A. cordifolia leaf decoctate at the onset of diarrhoeal stools showed a reduction in diarrhoeal episodes at doses of 100mg/kg, 200mg/kg and 400 mg/kg. The duration of diarrhoeal episodes over 6 hours of observation was 4.16 ± 0.70 hours, 2.5 ± 0.50 hours and 3.16 ± 0.70 hours respectively. In rats treated with loperamide 2mg/kg, diarrhoeal episodes lasted for 2.83 ± 0.70 hours. The mean weight of diarrhoeal faeces was calculated and shown in figure 2: 2277.83 ± 712.08 mg for the control group and 973.17 ± 272.22 mg for the standard drug group. Those of rats given A. cordifolia leaf decoctate were 1773.67±1722.5 mg, 1381.33±661.39 mg, 829.33±396.43 mg ($p = 0.003$) and 1135.17±212.87 mg. The respective percentages of reduction in diarrhoea (R) were 22.13% ($p>0.05$); 39.36% ($p = 0.04$); 63.59% ($p = 0.009$); 50.16% ($p = 0.04$) compared to control rats. That of loperamide was 57.28% ($p = 0.01$) **(Figure 2)**

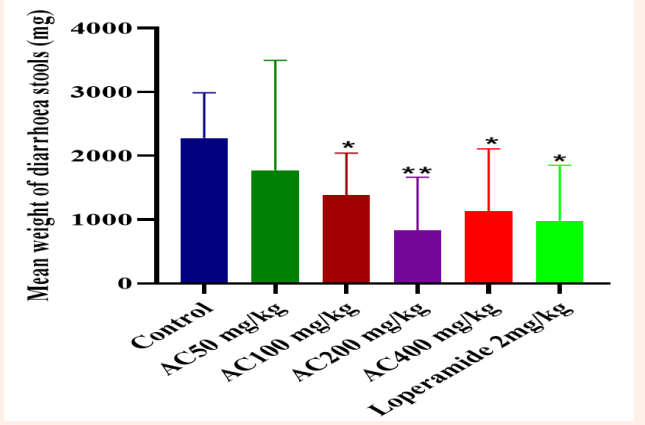

Figure 2. Weight of diarrhoeal faeces in rats receiving curative treatment

Wilcoxon test: *: $p<0.05$ compared with control; **: $p<0.01$ compared with control.

Discussion

The aim of this study was to assess the effect of an aqueous decoction of Alchornea cordifolia leaves in an animal model suffering from diarrhoea. Experimental diarrhoea was induced by castor oil. The ricinoleic acid contained in castor oil causes the release of prostaglandins responsible for the inflammation of the intestinal wall [11]. This irritation increases the permeability of enterocytes, leading to hypersecretion of electrolytes into the intestinal lumen, causing secretory diarrhoea [12]. Prostaglandins contribute to the pathophysiological functions of the gastro-intestinal tract [13]. When administered as a preventive measure, A. cordifolia leaf decoctate reduced the time of onset of diarrhoeal stools, the duration of diarrhoeal episodes and the mean weight of diarrhoeal stools. This activity was observed at a dose of 200 mg/kg where 4/6 rats did not produce any diarrhoeal faeces. The percentage of diarrhoea inhibition was 76.80% ($p = 0.003$). In the same animal model, Agbor et al. (2004) [6] also demonstrated that the preventive anti-diarrhoeal effect of an ethanolic extract of A. cordifolia leaves was at a dose of 800mg/kg with 64.65% diarrhoea inhibition. A. cordifolia leaf decoctate was found to be more effective. Joseph et al. (2015) [14] also demonstrated the preventive anti-diarrhoeal effect of an ethanolic extract and an aqueous extract of the stem bark of A. cordifolia was at a dose of 400mg/kg showing a better inhibition of diarrhoea, by 63.2% and 70.6% respectively. Heat extraction provides a better concentration of active ingredients. This method of preparation is also the preferred method of traditional healers. In curative administration of diarrhoea, A. cordifolia leaf decoctate showed a reduction in diarrhoeal episodes and the mean weight of diarrhoeal stools at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg with respective reduction percentages of 39.36% ($p = 0.04$); 63.59% ($p = 0.009$); 50.16% ($p = 0.04$). Under the same conditions, Agbor et al. (2004) [6] demonstrated the curative anti-diarrhoeal effect of an ethanolic extract of Alchornea cordifolia. The extract, administered at a dose of 400mg/kg bw to mice 15 minutes after receiving castor oil, reduced intestinal transit from 76.68 ± 7.24 to 56.33 ± 7.70, i.e. a percentage inhibition of 26.53%. Phytochemical screening of A. cordifolia leaf decocts revealed the presence of flavonoids, tannins, alkaloids, terpenoids and saponosides. These phytochemical groups could be responsible for this anti-diarrhoeal effect. The astringent properties of tannins tighten the pores in the intestinal mucosa, making them impermeable and reducing intraluminal hydroelectrolytic secretion [15]. Flavonoids modify the permeability of intestinal mucosa, inhibit intestinal motility and hydro-electrolytic secretion [16]. In addition, terpenoids and alkaloids inhibit the release of prostaglandins responsible for inflammation of the intestinal wall [17, 18] and reduce water secretion into the intestinal lumen. A. cordifolia leaf decoctate therefore has an anti-diarrhoeal effect. This effect could be due to the inhibition of the action of prostanglandins released by riciloleic acid. The decoction of A. cordifolia leaves could therefore lead either to a reduction in the volume of intestinal fluid, or an increase in the reabsorption of intraluminal fluid, or a slowing of intestinal transit (mechanism of action of loperamide), causing a reduction in the quantity of diarrhoeal faeces.

Conclusion

This study revealed the anti-diarrhoeal properties of Alchornea cordifolia leaf decoctate. The traditional use of this plant in the treatment of diarrhoea is therefore justified. However, the actual mechanism of action needs to be investigated.

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