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Safety, Tolerability and Anti-Diarrhoeal Activity of "Diarra", a Preparation of Medicinal Plants Used in Ivorian Traditional Medicine

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Abstract

Background: "Diarra", a traditional herbal remedy made from five (5) medicinal plants, might be endowed with anti-diarrhoeal properties according to its owner. However, scientific evidence of its safety, tolerability and activity has not been established. **Objective:** This study aimed to assess the safety, tolerability and anti-diarrhoeal activity of "Diarra" in experimental rats. **Materials and Methods:** Safety was assessed by acute (OECD 423) and sub-acute (OECD 407) toxicity studies at doses of 5, 10 and 20 mg/kg. Clinical tolerability was assessed for 28 days. On day 29, a blood sample was taken to evaluate biological tolerability. The anti-diarrhoeal activity was investigated in a castor oil-induced diarrhoea model. Rats were given the remedy at doses of 5, 10 and 20 mg/kg and then castor oil 1 hour later. They were observed for 4 hours and diarrhoeal stools were collected. The Percentage of diarrhoeal inhibition was calculated. **Results:** A single dose of "Diarra" at a dose of 2000 mg/kg did not induce any lethality, behavioural or weight change in rats for 14 days. When administered once daily for 28 days, "Diarra" did not cause lethality or significant behavioural disorders or significant weight loss in rats. No biological disorders were observed. The treatment of rats with "Diarra" at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg in a single administration inhibited the occurrence of diarrhoeal stools. The respective percentages of inhibition were 60%, 50% and 62%, similar to those of loperamide at a dose of 2 mg/kg (68%). **Conclusion:** "Diarra" has an anti-diarrheal activity in rats. It is also safe to use this remedy as such.

Keywords

Safety, Tolerability, Antidiarrheal, Diarra, Traditional Medicine

1. Introduction

Traditional medicine is becoming a better therapeutic alternative given the socio-economic and affordability of conventional medicine issues. More than 80% of the African population relies on traditional medicine for their healthcares [1]. This medicine is full of remedies which secret was held by traditional healers for several generations [2]. Moreover, since 1977, WHO has paid a particular attention to traditional medicine as it helps to achieve the goal of providing access to healthcare for all [3]. Among remedies offered by traditional medicine, we have "Diarra", a preparation of medicinal plants from ancestral recipes used as an anti-diarrheal and property of Dr. Fofana Mamadou Yacine, pharmacist and traditional healer. Diarrhoea is indeed a transit disorder characterised by loose or even liquid stools, in abnormally high quantity or with an increased frequency of occurrence and several times a day [4]. Diarrhoeal diseases cause about 1.8 million deaths worldwide annually and 90% are children under the age of five, living mostly in developing countries [5]. Diarrhoeal diseases are the third cause of death from infectious diseases for all ages [6] and the fifth leading cause of premature death globally [7].

The general objective of this study was to investigate the safety, tolerability and anti-diarrhoeal activity of "Diarra" in experimental rats.

2. Materials and Methods

2.1. Experimental Animals

Forty (40) Wistar rats (*Rattus norgevicus*) of both sexes, between 2 to 3 months of age and weighing between 150 and 250 g were obtained from the laboratory of pharmacology, faculty of pharmaceutical and biological Sciences, University of Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire. Animals were kept under a standard condition room of 24 ± 1˚C with 12 hours of light and dark cycle. Animals had free access to water and food. They were fed with standard food pellets supplied by Ivograin®. Prior to the experiment, animals were kept fasting for 12 hours with free access to water.

2.2. The Remedy "Diarra"

The remedy to be investigated in this study is called "Diarra". "Diarra" is a preparation of medicinal plants in the form of dry powder, green in colour with a characteristic odour. The powder has a slightly sweet taste with a bitter aftertaste. This remedy is the property of Dr Fofana Mamadou Yassine, pharmacist and founder of "Laboratoire Galefomy " located in Bouaké (Côte d'Ivoire) and known by the national program for the promotion of traditional medicine. "Diarra" is a mixture of five (5) medicinal plants packaged in a white bottle of 50 g (**Figure 1**). According to the owner's recommendations, this remedy has anti-diarrhoeal properties and should be used at a dosing of one (1) teaspoon of powder boiled in 250 ml of water, filtered and be consumed day and night. The medicinal plants used for this remedy are *Euphorbia hirta* (Euphorbiaceae) L., *Mangifera*

Figure 1. Vial of the "Diarra" remedy.

indica (Anacardiaceae) L., *Erythrina senegalensis* (Papilionaceae) L., *Oriza sativa* (Poaceae) L. and *Adansonia digitata* (Combretaceae) L.

2.3. Technical Equipment

The technical equipment were syringes, feeding tubes, blood collection tubes with red and purple caps, precision scales (OHAUS®), cotton wool, watch glasses, Pasteur pipettes, laboratory notebook, examination gloves, markers, compresses, beakers, centrifuge (Memmert®), spatulas, pipettes (1 ml, 2 ml, 5 ml and 10 ml) and metabolic cages.

2.4. Solvents and Reagents

For this study distilled water, ether, tap water, normal saline (0.09%), castor oil (Dermophar, Ivory Coast), loperamide (Diarum®), ether (Gifrer®) were used as solvents and reagents.

2.5. Calculating the Weight Corresponding to a "Teaspoon"

Prior to drug administration, instructions for the product preparation were followed as written on the bottle. The quantity corresponding to one teaspoon was determined. To do this, a teaspoon of the product was weighed 10 times and the mean was calculated.

2.6. Determination of Test Doses

2.6.1. Determination of the Dry Matter Concentration of "Diarra"

A teaspoon of "Diarra" was boiled in 250 ml of water for 5 min. The decoction was filtered. The filtrate (170 ml) was dried in an oven at 40˚C for 24 h. The dry matter obtained was weighed (1353 mg), providing a drinkable concentration of 7.95 mg/ml or about 8 mg/ml.

2.6.2. Determination of Doses to Be Tested in Experimental Animals [8]

For an adult weighing 70 kg, 1353 mg of a drinkable solution of "Diarra" corresponds to a dose of 19.32 mg/kg. For a child weighing 10 kg, this quantity corresponds to 135.3 mg/kg. Conversions to equivalent doses in experimental animals gave doses of 2.89 mg/kg and 20 mg/kg, respectively. Three doses of "Diar-

ra" were tested in three (3) groups of six (6) rats each: 5 mg/kg, 10 mg/kg and 20 mg/kg.

2.7. Preparation of "Diarra" Solution

Thirty-two (32) mg of the dry decoctate obtained after extraction was dissolved in 16 ml of normal saline, which enable us to get 16 ml of a concentrated stock solution of 2 mg/ml. An administration of 10 ml/kg of this solution corresponds to a dose of 20 mg/kg (Diarra 20). The stock solution was diluted at a rate of 1/10 using the method of double dilution in geometric progression to respectively obtain two (2) solutions at 10 mg/ml (Diarra 10) and 5 mg/ml (Diarra 5).

2.8. Acute Toxicity of "Diarra" (OECD 423)

In this study, six (6) female wistar rats were used as experimental animals. Animals were divided into two (2) homogeneous groups of three (3) rats each.

- Group 1: rats were given normal saline by oral route at a volume of 10 ml/kg b.w.
- Group 2: rats were administered "Diarra" by oral route at a dose of 2000 mg/kg in a volume of 10 ml/kg.

Rats were carefully observed for 4 hours and then every day for 14 days for lethality and/or signs of clinical toxicity (apathy, excitement, breathing disorder, excessive grooming, refusal to eat or drink, oral or nasal bleeding, abdominal pain, coma, diarrhoea, tremors, convulsions, death). Furthermore, animals were weighed every day.

2.9. Subacute Toxicity of "Diarra" (OECD 407)

Forty (40) male and female Wistar rats were used as experimental animals. Animals were divided into 4 groups of 10 animals each (5 females and 5 males) and received once daily by oral route at a volume of 10 ml/kg for 28 days: group 1, was given normal saline (NaCl); group 2, received "Diarra" at a dose of 5 mg/kg; group 3, was administered "Diarra" at a dose of 10 mg/kg; group 4, was given "Diarra" at a dose of 20 mg/kg.

Rats were daily weighed and examined for clinical signs of toxicity. On day 29, animals were anaesthetised with ethyl ether. A blood sample was taken by puncturing the retro orbital sinus and collected in two tubes, one containing EDTA (purple tube) and the other red dry tube. The blood from the EDTA tubes (purple tube) was used for haematological analysis (CBC), whereas the one from the red tubes was centrifuged at 4000 rpm for 10 minutes. The serum was collected and stored at −20˚C for further assays to explore renal (urea, creatinine), hepatic (AST, ALT) and pancreatic (glycaemia) functions.

2.10. Antidiarrhoeal Activity of "Diarra

2.10.1. Principle

Castor oil administered orally causes experimental diarrhea [9]. An antidiarr-

hoeal agent is thought to reduce the volume of intestinal fluid, either by increasing the reabsorption of intraluminal fluid or by slowing intestinal transit, by reducing the amount of diarrheal stools. Loperamide, a slower of intestinal transit was used as a positive control.

2.10.2. Procedure

Thirty (30) rats, kept fasting for 12 hours, were divided into 5 homogeneous groups of six (6) rats each and given by oral route 10 ml/kg: group1, received normal saline (NaCl); group 2, was administered loperamide at a dose of 2 mg/kg; groups 3, 4 and 5 were given "Diarra" at a respective dose of 5 mg/kg, 10 mg/kg and 20 mg/kg.

One (1) hour later, diarrhoea was induced by oral administration of castor oil to each rat and was immediately placed in a metabolic cage and observed for 4 hours. The characteristic diarrhoeal stools were collected in a bottle placed under the individual perforated cages of rats. The mean weight of diarrhoeal stools from each group was determined 5 h after induction of diarrhoea by castor oil.

The percentage of diarrhea inhibition was calculated using the following formula:

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

I(%): percentage of diarrhea inhibition.

M: mean weight (mg) of diarrhoea stools in control group.

m: mean weight (mg) of diarrhoea stools in treated group.

2.11. Data Processing and Analysis

The results were expressed as a mean ± standard deviation. Graphs were performed using Graph Pad Prism software 8.0.2. Means were compared using the Wilcoxon Test at a risk of 5%.

3. Results

3.1. Acute Toxicity

A single administration of "Diarra" at a dose of 2000 mg/kg did not cause any lethality, behavioural disorders or significant weight loss in rats. Indeed, from day 1 to day 9, the mean weights of rats increased from 182.3 ± 15.19 mg to 191.7 ± 14.77 mg for rats treated with normal saline and from 183.3 ± 9.025 mg to 194.0 ± 8.737 mg for rats given the single dose of "Diarra". From day 9 to day 12 a weight constancy were observed in rats of both groups (**Figure 2**) ($p > 0.05$).

3.2. Subacute Toxicity

"Diarra" administered once daily at doses of 5, 10 and 20 mg/kg for 28 days did not cause any lethality, behavioural disorders or significant weight loss in rats (**Figure 3**).

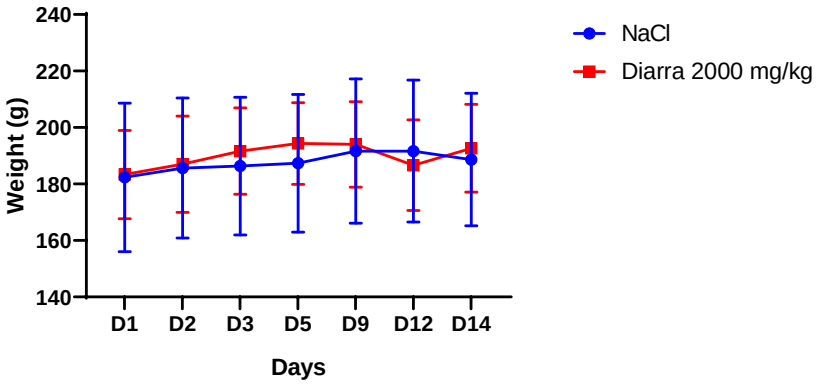

Figure 2. Evolution of the weight of rats having received "Diarra" at 2000 mg/kg.

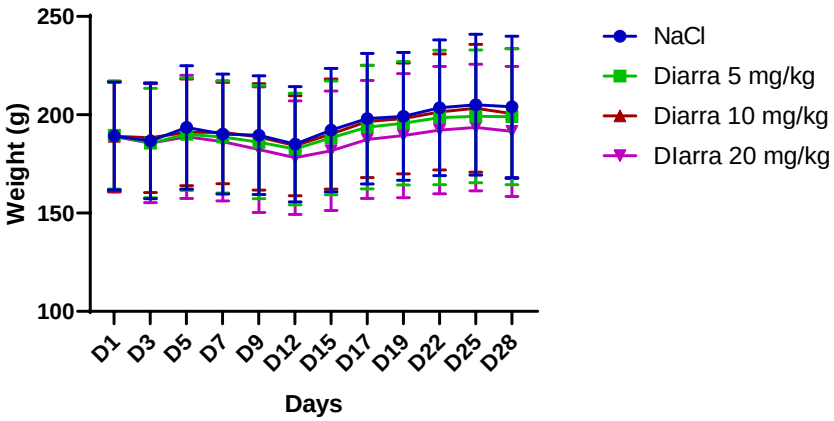

Figure 3. Evolution of the weight of the rats having received the "Diarra" sample in a single daily administration over 28 days.

3.2.1. Effects of "Diarra" on Biochemical Parameters

Figure 4 shows the effect of "Diarra" on uraemia (A) and creatinemia (B). It shows that the administration of "Diarra" at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg for 28 days did not cause any significant changes in uraemia and creatinaemia.

Figure 5 shows the effect of "Diarra" on blood glucose levels. After 28 days of daily administration of "Diarra" at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg, there was no significant change in blood glucose levels.

Figure 6 shows the effect of "Diarra" on the activity of transaminases: ALT (A) and AST (B); where no change was observed after administration of "Diarra" at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg.

3.2.2. Effects of "Diarra" on Hematological Parameters

Figure 7 shows the effect of "Diarra" on white blood cell count (A), red blood cell count (B), haemoglobin (C) and platelet count (D). No significant change was observed in these parameters ($p > 0.05$). The other haematological parameters (haematocrit, mean corpuscular volume, mean corpuscular haemoglobin content, lymphocytes monocyte, polynuclear neutrophils, polynuclear eosinophils, polynuclear basophils) did not show any significant change as well ($p > 0.05$).

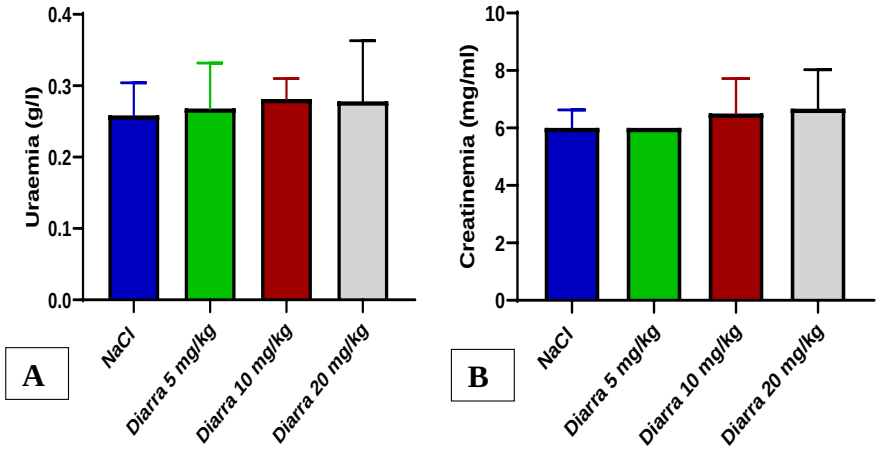

Figure 4. Effect of "Diarra" on uraemia and creatinimia.

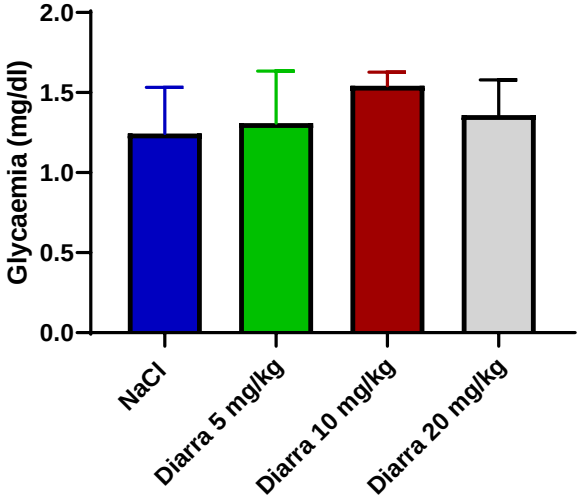

Figure 5. Glycaemia of rats after 28 days of exposure to the "Diarra" sample in a single daily administration.

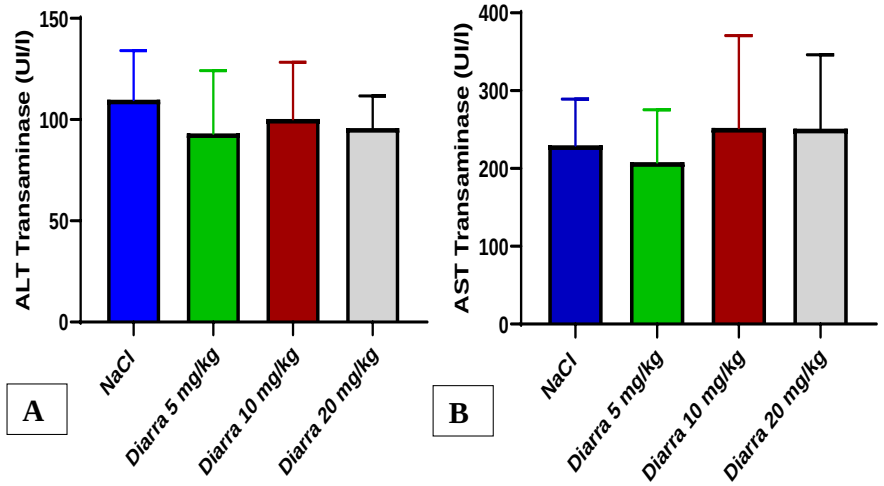

Figure 6. Transaminase activity of rats after 28 days of exposure to "Diarra" in a single daily administration.

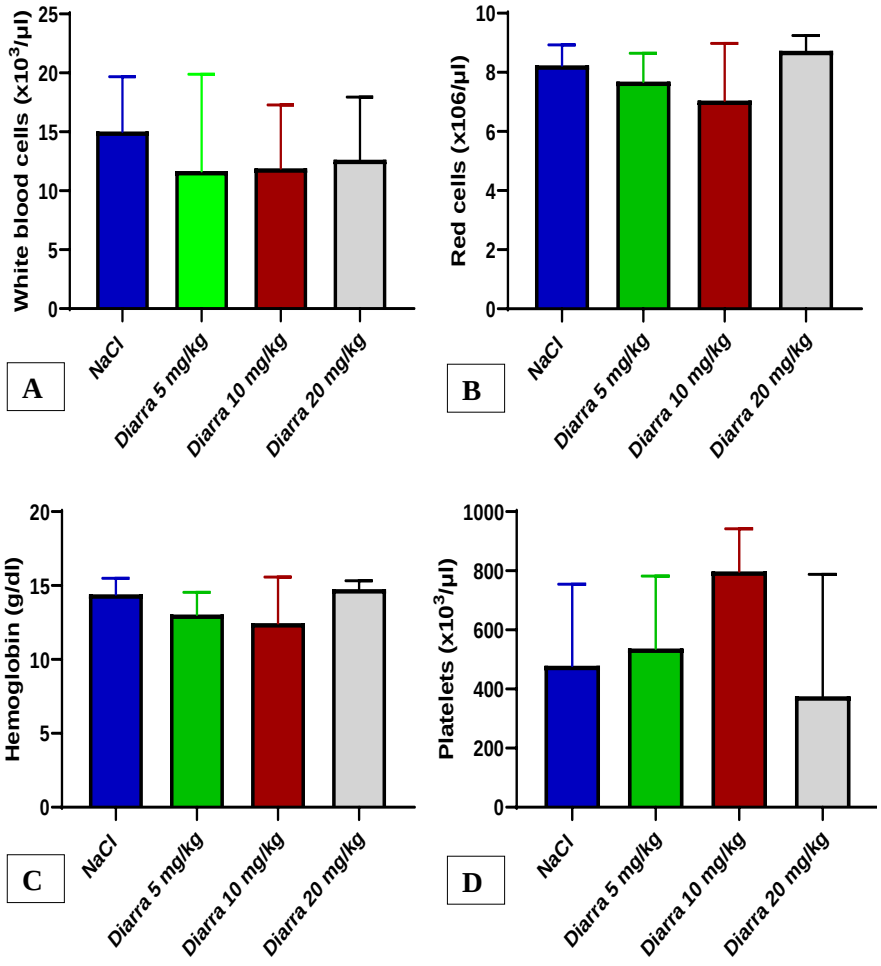

Figure 7. Values of some hematological parameters of rats after 28 days of exposure to "Diarra" in single daily administration.

3.3. Anti-Diarrhoeal Activity

A single administration of "Diarra" at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg significantly inhibited ($p < 0.0005$) the occurrence of diarrhoeal stools. The respective percentages of inhibition were 60%, 50% and 62%, similar to that of loperamide at a dose of 2 mg/kg which inhibited diarrhoeal stool at a rate of 68% ($p > 0.05$). Indeed, the mean weights of diarrhoeal stools were 1729 ± 121.7 mg for rats treated with normal saline, 686.67 ± 21.73 mg, 856.67 ± 20.30 mg and 643.33 ± 22.16 mg for rats given the respective doses of "Diarra" and 539.17 ± 18.09 mg for rats administered with loperamide at a dose of 2 mg/kg as shown in Figure 8. The doses used in a single administration did not show dose-dependent activity.

4. Discussion

This study aimed to investigate the safety, tolerability and anti-diarrhoeal activity of "Diarra", a preparation medicinal plant used in Ivorian traditional medicine, in experimental rat.

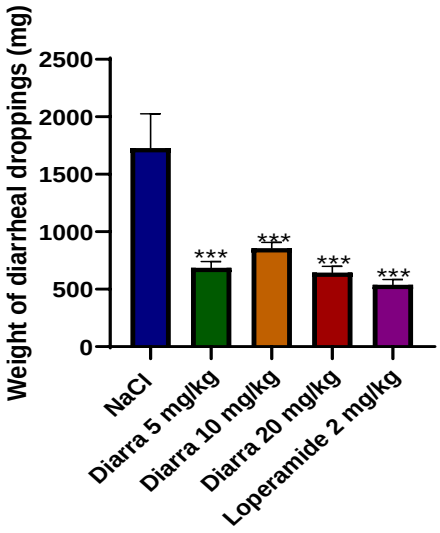

Figure 8. Average weight of diarrheal droppings of rats.

4.1. Toxicity of "Diarra"

The administration of "Diarra" at a single dose of 2000 mg/kg and after observation of 14 days, did not induce any lethality, nor any behavioural disorders or weight change in female rats. Also, when administered once daily for 28 days, "Diarra" at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg did not cause any lethality, behavioural disorders or weight gain in rats. In addition, there were no significant changes in some parameters assessing renal (urea, creatinine), pancreatic (blood glucose), hepatic (AST, ALT) or haematological (CBC) functions. Indeed, several researches have reported the absence of toxicity from each of the investigated plants. Thus, *Euphorbia hirta* is not toxic to humans [10] [11]. For *Erythrina senegalensis*, a toxicological study carried out in Côte d'Ivoire showed that the LD_{50} was 1633 mg/kg and 1770 mg/kg body weight using the methods of Miller-Tainter and Dragsted-Land [12]. "Diarra" at a dose of 2000 mg/kg b.w contains five (5) medicinal plants and therefore a lower dose of *Erythrina senegalensis*, which could explain the absence of toxicity.

Mangifera indica, *Oriza sativa* and *Adansonia digitata* are food plants whose toxicity has not been reported. In addition, their use in combination with other medicinal plants is done at a low dose.

4.2. Antidiarrhoeal Activity of "Diarra"

The anti-diarrhoeal activity of "Diarra" was assessed using the castor oil-induced diarrhoea method. Orally administered, castor oil causes experimental diarrhoea. The ricinoleic acid contained in castor oil irritates the intestinal mucosa and increases its permeability, which explains the hypersecretion of fluid in the intestinal lumen, loosening the stools [13]. An antidiarrhoeal agent could reduce the volume of intestinal fluid, either by increasing the reabsorption of intraluminal fluid, or by slowing intestinal transit, the consequence being a reduction in the amount of diarrhoeal stools.

"Diarra" at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg, in a single administration inhibited the occurrence of diarrhoeal stools with respective percentages of inhibition of 60%, 50% and 62%. Diarra" has a preventive anti-diarrhoeal activity, which comes from the anti-diarrhoeal properties of the medicinal plants it contains.

Euphorbia hirta is used traditionally in the Central African Republic as an antidiarrhoeal [14]. This activity has been demonstrated with freeze-dried decoctions on intestinal transit. Quercitrin, a flavonic compound, might be involved in this effect [15]. Other studies showed that *E. hirta* has an *in vivo* anti-diarrhoeal effect [16]. This anti-diarrhoeal activity could also be explained by its content in tannins. An ethanolic extract of *Euphorbia hirta* has been shown to have a healing effect on wounded rats [17]. As for *Mangifera indica*, various organs, such as leaves, stems, bark and seeds, are rich in tannins [18]. In Senegal, various astringent preparations of the bark and leaves are used to treat diarrhoea. In Niger, the roots are used to treat diarrhoea [19] [20]. The potential anti-diarrhoeal activity of methanolic and aqueous extracts of *Mangifera indica* seeds was accessed in a model of experimental diarrhoea induced by castor oil and magnesium sulphate in mice. The results exhibited a significant anti-diarrhoeal activity [21]. Concerning *Erythrina senegalensis*, the leaves are used to treat gastrointestinal disorders in traditional medicine [22]. The anti-diarrhoeal activity of this plant was evaluated *in vivo* by inducing diarrhoea with castor oil in rats and *in vitro* using isolated rabbit jejunum. *Erythrina senegalensis* (500 and 1000 mg/kg) significantly reduced ($p < 0.05$) the frequency of diarrhoeal stools and the spontaneous propulsive movement of isolated rabbit jejunum (anti-motility) [23]. For *Oriza sativa*, the varieties of this medicinal plant contain phytochemical compounds such as saponins, terpenoids and tannins [24]. Diarrhoea is one of the diseases for which rice is traditionally indicated [25]. Rice is used to treat diarrhoea in children. A teaspoon of carbonised baked rice powder mixed with a glass of butter-milk should be given in single doses (about 28 g) every half hour. This will bring out excellent results [25].

Finally, *Adansonia digitata* is used for the preparation of numerous remedies in traditional African pharmacopoeia, particularly for digestive and inflammatory issues [26] [27] [28] [29]. Although edible, the dried pulp of the fruit (bouye juice) in the form of a decoction is used in West and Southern Africa as an anti-diarrhoeal for its astringent properties. The pulp, which also heals and strengthens children, is said to treat diarrhoea, dysentery and inflammation of the intestine and liver [28]. *Adansonia digitata* contains tannins which may justify the use of the seed and pulp as an anti-diarrhoeal [30].

"Diarra" therefore has anti-diarrhoeal properties due to each of the medicinal plants it contains.

5. Conclusions

The remedy "Diarra" in a single administration was not toxic up to 2000 mg/kg.

When administered once daily for 28 consecutive days, "Diarra" at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg showed no clinical signs of toxicity, nor any disorders in blood parameters, reflecting damage of some internal organs in experimental rats, in particular the kidneys, liver, pancreas and haematological parameters. These doses significantly reduced the volume of intestinal fluid generated by castor oil. The mechanism of this anti-diarrhoeal activity is either an increase in the reabsorption of intraluminal fluid, or a slowing in intestinal transit, causing a reduction in the quantity of diarrhoeal stools.

"Diarra" therefore has an anti-diarrhoeal activity in rats. It is also safe to use this remedy as such.

Conflicts of Interest

The authors declare no competing interests in this study.

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