ANTIDIARRHOEAL ACTIVITIES OF AQUEOUS AND METHANOLIC EXTRACTS OF MANGIFERA INDICA LINN STEM BARK (ANARCADIACEAE) IN WISTAR RATS

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Abstract

Diarrhea is one of the leading causes of infant mortality in developing country. It has been reported that *Mangifera indica (Mi)* is used as an antidiarrhoeal agent in traditional medicine. This study was to evaluate the antidiarrhoeal activities of aqueous and methanolic extracts of *Mi* Linn stem bark. The extracts were tested *in vivo* at doses of 300, 400 and 500 mg/kg on the effects of *Escherichia coli,* castor oil, misoprostol and charcoal meal in rats. *In vivo*, aqueous extract at doses of 300 and 500 mg/kg and methanolic extract at dose of 500 mg/kg resulted in a significant decrease ($p < 0.001$) in the bacterial load in rats faeces. A significant reduction ($p < 0.001$) of diarrhoeal parameters was observed with both extracts after castor oil administration. The gut motility was significantly ($p < 0.05$) reduced with all doses of methanolic extract. Histopathological analysis revealed few abnormalities in structure and morphology of the ileum after treatment of rats with aqueous and methanolic extract This study showed that *Mi* possesses antidiarrheic properties and attest its usefulness in traditional treatment of gastrointestinal infection.

Keywords: *Mangifera indica* Aqueous extract Methanolic extract Antidiarrheal.

Introduction

Defining as frequent emission of liquid stools, diarrhea involves an increase in volume, frequency and fluidity of stools with a loss of electrolytes and water (Neelam *et al*. 2014). It can be caused by poor digestion, infectious disorders, and malabsorption, (Hanwa *et*

al. 2007). Diarrhea can be acute (2 weeks), persistent (2 - 4 weeks), in this case it is caused by enteric pathogens, or chronic when it lasts more than 4 weeks and in this case it is caused by malabsorption syndromes, and inflammatory disorders (May and Chisholm-Burns 2016).

Worldwide, according to the WHO report (Shrivastava *et al*. 2017), diarrhea is the leading cause of death in children under 5, and the third cause of death at all ages (then represent the main causes of morbidity and mortality (Broder *et al*. 2016). In addition, diarrhea is responsible for almost 80% children deaths in developing countries. In sub-Saharan Africa, 39.1% of the population is likely to develop diarrhea each year; while in Cameroon, 21% of children under 5 years develops diarrhea (UNICEF, 2012).

The modern treatment of diarrhea includes loperamide, diphenoxylate and antibiotics is a non-specific treatment; it aims to reduce the frequent bowel movements and the discomfort (Neelam *et al*. 2014); in addition, these treatments lead to the development of side effects (Anup *et al*. 2007). Although synthetic products are easy to use and inexpensive, more than 90% of rurals populations depend on medicinal plants to treat diarrhea (Ahmadu *et al*. 2007). WHO (2013) reports that more than 80% of the population of African countries use the pharmacopoeia and herbal medicine to deal with their health problem (Mangambu *et al*. 2012); thus medicinal plants represent an invaluable hope in the treatment of many pathologies. Some plants have already shown significant antidiarrheal activities, as *Sapium Ellipticum* (Wansi *et al*. 2014), *Mangifera indica* (Anacardiaceae), a tree found in tropical and subtropical regions, is widely used in Cameroon to treat diarrheal diseases. In traditional practice, almost all parts of this plant are used to treat various pathologies such as diabetes, viral diseases, inflammatory pathologies, diarrhea, hypertension, and many others (Khyati *et al*. 2010). Several classes of compounds with pharmacological properties have also been found in extracts from this, namely flavonoids, polyphenols, triterpenoids (Khyati *et al*. 2010. Likewise, pure compounds have also been isolated from this plant, this is the case for mangiferin, catechin, shikimic acid, n-triacontane and mangiferolic of the acidic methyl ester (Aderibigbe *et al*. 2001). Several polyphenols such as kaempferol, gallic acid, quercetin, have also been found in this extracts (Singh *et al*. 2004). the aim of this work were to evaluate

the *in vivo* anti-diarrheal activities of the aqueous and methanolic extracts of the stem barks of *Mangifera indica* harvested in the region of West Cameroon.

Materials and methods

Drugs and chemicals

Atropine sulfate was obtained from Sigma Aldrich (St. Louis, MO63103 USA). Castor oil, methanol, distilled water were obtained from Geochim, sodium chloride (Sigma Chemicals Co., UK), activated charcoal flour (Carbophos E153) from Tradiphar (Lille, France); Loperamide obtained from Biotech Co, Ltd (Shaanxi, China).

Plant material and extraction

The stem barks of *M. indica* were collected in West Region, Cameroon in the plant was authenticated at number N° 18646/HNC. Fresh *M. indica* stem bark was air-dried at room temperature (24–25 °C) out of direct sunlight and ground to a fine pow der using an electric mixer. This powder was used for aqueous and methanolic extractions.

The extraction was carried out by maceration of 500 g of *M. indica* stem bark powder in 5 L of distilled water for 72 h. The mixture was filtered using Whatman filter paper (n° 1) and the filtrate was dried in an oven set at 40° C for a few days. After all, 115.3 g of aqueous extract were obtained.

Concerning the methanolic extract, 500 g of the powder were macerated in 3 L of methanol (99%) for 24 h. The mixture was filtered and the filtrate was concentrated under reduced pressure with a rotary evaporator at 65 °C. This process gave 71.745 g of methanolic extract corresponding to a yield of 14.3% extraction.

Qualitative phytochemical tests

The qualitative determination of alkaloids, flavonoids, phenols, saponins, sterols, tannins and triterpenes in the aqueous and methanolic extracts of *M. indica* was carried out according to the standard protocols described by Palombo (2006).

In vivo anti-diarrheal test

Animals

For this work, young (1 month) albino rats of *Wistar* strain of both sexes were used. They were raised in the animalery of the University in conditions of natural light with free access to drinking water and food.

The *in vivo* protocols have been accepted by the laboratory committee and carried out in accordance with the European Union directives for the protection of animals (CEE Council 86/609).

Castor oil-induced secretory diarrhea

Secretory diarrhea was induced using beaver oil according to the method described by Memi *et al.* (2017) with slight modifications. Forty-eight (48) fasted for 18 h were divided into 8 groups of 6 rats each. Group 1 was treated with distilled water (10 mL/kg, *p.o.*) and was considered as the negative control; group 2 (positive control) was treated with atropine sulfate (3 mg/kg, p.o.) as standard drug; groups 3, 4 and 5 received the aqueous extract of *M. indica* (AEMI) (300, 400 and 500 mg/ kg, respectively); groups 6, 7 and 8 received the methanolic extract of *M. indica* (MEMI) (300, 400 and 500 mg/ kg, respec tively). One hour after treatment, all the animals received orally 1 ml of beaver oil and were distributed individually in a partitioned wire cage, with filter paper placed below. Each rat was observed for 6 h and the time of onset of diarrhea, the frequency of diarrheal stools, the total emission of stools and the stool water content were recorded. The percentage inhibi tion (% I) of diarrhea was calculated as follows

$$\% \text{ inhibition} = x = \frac{(\text{Wet defection})\text{Control} - (\text{Wet defection})\text{Test}}{(\text{Wet defection})\text{Control}} \times 100$$

Gastrointestinal motility test with charcoal flour

The motility test was carried out according to the method described by Bellah *et al*. (2014) with slight modifications. For this test, the animals (48) left on an empty stomach for 24 h were distributed and treated as in the previous test, except that for this test atropine sulfate (5 mg/kg, *i.p.*) was used as reference drugs. One hour after administration of various treat ments, each animal received orally 1

ml of Beaver oil and 30 min after, each animal received orally 1 ml of charcoal flour (10% deactivated in distilled water; w/v); 30 min later, they were sacrificed after injection 0.5 mL of diazepam (10 mg/kg, *i.p.*)/ketamine (50 mg/kg, *i.p.*), then the section of the small intestine from the pylorus to the cecum was sampled, deployed and the distance traveled by the charcoal was measured. The peristaltic index (PI) was calculated based on its relationship to the total length of the small intestine of each rat as follows:

$$\text{Peristaltic index(PI)} = \frac{\text{Distance traveled}}{\text{Length of full bowel}} \times 100$$

The percentage inhibition (% I) was calculated as follows

$$\%\ \text{inhibition} = x = \frac{(\text{PI})\text{Control} - (\text{PI})\text{Test}}{(\text{PI})\,\text{Control}} \times 100 x = \frac{(\text{PI})\text{Control} - (\text{PI})\text{Test}}{(\text{PI})\,\text{Control}} \times 100$$

Enteropathic Escherichia coli induces infectious diarrhea

The model of infectious diarrhea caused by *Escherichia coli* was carried out according to the modified protocol of Ngoudjou *et al*. (2017). After acclimatization of the animals under sterile and aseptic conditions, they disinfected by oral administration of tetracycline (10 mg/kg). The colony count of *E. coli* species of the microbiota was carried out after daily cultivation of rat feces.

Fifty-four (54) rats were divided into 9 groups of 6 rats each. Group 1 served as the neutral control and was made up of uninfected and untreated rats; group 2 or negative control group was infected and treated with distilled water; group 3 (positive control) was infected and treated with ciprofloxacin (3 mg/kg, *p.o*) as standard drug; groups 4, 5 and 6 were infected and received the AEMI (300, 400 and 500 mg/kg, respectively); groups 7, 8 and 9 were infected and received MEMI (300, 400 and 500 mg/kg, respectively). Each treatment was administered after induction of infectious diarrhea with an enteropathogenic *Escherichia coli* strains ATCC 8739 inoculum (2 mL), prepared in autoclaved 0.9% NaCl by collecting refreshed bacteria on a Mueller–Hinton Agar II (MHA II) culture, according to the 0.5 McFarland standard scale (1.5 × 108 CFU/mL). The number of bacteria was determined daily in 0.5 g of faeces samples dis-

solved in 5 ml of sterile physiological saline. After homogenization, 50 µL of the supernatant was distributed over the surface of the Petri dishes filled with Sorbitol MacConkey Agar (SMA). The cultures were therefore incubated at 37 °C. for 24 h, after which the number of EPEC colonies was recorded and definitively expressed in colony forming units per gram of faeces (CFU:g of faeces). The relative body weight of the animals was also determined during the treatment period. At the end of the experiment, the animals were sacrificed; the ileum was collected in a 10% formaldehyde solution for a histopathological study according to standard methods

Statistical analyzes

The data were analyzed using Graphpad prism 5.03 software. One-way ANOVA followed by the Tukey post-test was used for antisecretory and anti-accumulative experiments, while two-way ANOVA followed by the Bonferroni post- test was used for the variation of the bacterial load of the faeces, the change in body weight in the induced infectious diarrhea test and in the motility test. All data were expressed as mean ± standard error of mean (SEM) and determined to be significant at $p < 0.05$.

Discussion

This study aims to evaluate antidiarrhoeal effects of aqueous and methanolic extracts of *M. indica* via *in vivo* studies. The *in vivo* results show that *M. indica* have antidiarrheal activity using diarrheal models induced by enteropathogenic *Escherichia coli* (Figure 1), Castor oil (Table 2, Table 3) ,

Castor oil-induced diarrhea is a model used to assess the antisecretory potential of pharmacological substances. Ricinoleic acid (12-hydroxyoleic acid) is a compound released into the intestinal lumen during hydrolysis of Beaver oil lipases. It is capable of inducing changes in permeability in the mucosa, transport of electrolytes and intestinal peristalsis which lead to a hypersecretory response and to diarrhea (Mebude *et al*. 2017). This compound also has a mucosal inflammatory effect by increasing the permeability of intestinal epithelial cells, thereby stimulating the release of E-series prostaglandins (PGE). These PGEs can therefore also be linked to seven receptors coupled to rembopsin-type transmembrane G proteins

(GPCRs) (and induce all the cascade reactions that activate the contractions of the intestinal smooth muscles (Pintać *et al*. 2018), or increase the secretion of and electrolytes in the small intestine. In this study, the extracts and atropine sulfate (Table 2) considerably reduced ($p < 0.001$) the time of onset of diarrhea, the frequency of diarrheal stools and the total number of stools produced after 6 h of observation. Since the extracts effectively inhibited diarrhea induced by beaver oil, it can therefore be assumed that the antidiarrheal action would also be mediated by an antisecretory mechanism. This was also evident from the decrease in the total number of wet feces in the test group in the experiment (Raj *et al*. 2017). In vivo experiments have shown that flavonoids are capable of inhibiting the intestinal secretory response induced by prostaglandins E2 (PGE2) (Sanchez de Medina *et al*. 1997), flavonoids have antioxidant properties which are presumed to be responsible for the inhibitory effects exerted on several enzymes, including those involved in the metabolism of arachidonic acid (Mora *et al*. 1990). Flavonoids also have the ability to inhibit intestinal motility and the hydroelectrolytic secretions that are altered in this intestinal condition (Rao *et al*. 1997).

The extracts showed significant antidiarrheal activity against gastrointestinal motility (Table 3). Several studies have shown that activated charcoal adsorbs drugs and chemical components sufficiently on the surface of charcoal flour particles, thereby preventing absorption (Levy 1982). Therefore, a gastrointestinal motility test with activated charcoal was performed to find out the effect of M. indica extracts on the movement of peristalsis. The results (Table 3) of this test also show that the extracts suppressed the propulsion of charcoal flour, thereby increasing the absorption of water and electrolytes (Raj *et al*. 2017). The presence of triterpenes in the different extracts (Table 1) is responsible for the activity of this plant; since some triterpenes have the ability to reduce the propulsive movement of charcoal flour, thus showing inhibition of gastrointestinal motility comparable to that of atropine sulfate (a muscarinic receptor blocking agent) (Bibi *et al.* 2015).

Concerning infectious diarrhea, recently, studies have reported that entero-pathogenic *E. coli*, the producer of Shiga toxin, entero-aggregative and, in some cases, enterotoxigenic, are the most common clinically based pathotypes in childhood diarrhea (Petri *et*

al. 2008). In fact, by releasing enterotoxins, they generate inflammations and localized lesions of the intestinal wall. It therefore loses its absorption capacity and becomes the site of hypersecretion which leads to dysentery (Tapia *et al*. 2017). The results revealed that the aqueous and methanolic extracts of *M. indica* exerted a significant inhibitory effect against the growth of fecal EPEC (Figure 1). In addition, the reduction in the bacterial load observed from the 4th day in all the faeces of infected animals treated with the extracts, could demonstrate their bactericidal effect. It has been shown that certain classes of secondary metabolites can also express their antibacterial potential by denaturing the membrane of bacterial lipopolysaccharides (LPS) specifically that of gram-negative bacteria such as *E. Coli* species. The micrographs of the ileum (Figure 2) obtained from our study showed an overall epithelial regeneration, when the animals received a treatment with aqueous and methanolic extracts *M. indica*. Since extracts of *M. indica* contain flavonoids, alkaloids and tannins which, by their anti-inflammatory properties, can play an important role directly or indirectly in the treatment of diarrhea (De Santana *et al*. 2017). These compounds present in the extracts (Table 1) may therefore have favored the activation of the signaling pathways, thus facilitating the tissue reconstitution observed in the mucosa of the ileum.

Conclusion

These data essentially demonstrated the anti-diarrheal effects of aqueous and methanolic extracts of *M. indica* stem bark. These effects could be mediated by antidiarrheal pathways and could be due to the presence of secondary metabolites present in the plant. The results therefore correspond to the claims of traditional healers and this species is therefore a good candidate for the development of new phyto-drugs.

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Table 1: Preliminary phytochemical study of aqueous and methanolic extracts of Mangifera indica stem bark.

Class of secondary metabolites	Tests	Aqueous Extract	Methanolic Extract
Polyphenols	Ferric chloride	+	+
Flavonoids	Shinoda/Alkaline reagent	+	+
Triterpenes	Liebermann Burchard	+	+
Steroids	Liebermann Burchard	-	-
Saponins	Frothing/Foam	-	+
Alkaloids	Dragendroff/Mayer	+	-

+ : Présent ; - : Absent

Table 2: Antisecretory effects of aqueous and methanolic extracts of *M. indica* on castor oil induced diarrhoea in rats.

Treatment	Doses (mg/kg)	onset (min)	Diarrhoeal stools frequence	Inhition (%)	Total stools frequence	Water content (%)
Distilled water	1mL/100 g	50.17 ± 2.21	3.83 ± 0.40	-	4.33 ± 0.42	83.06 ± 3.86

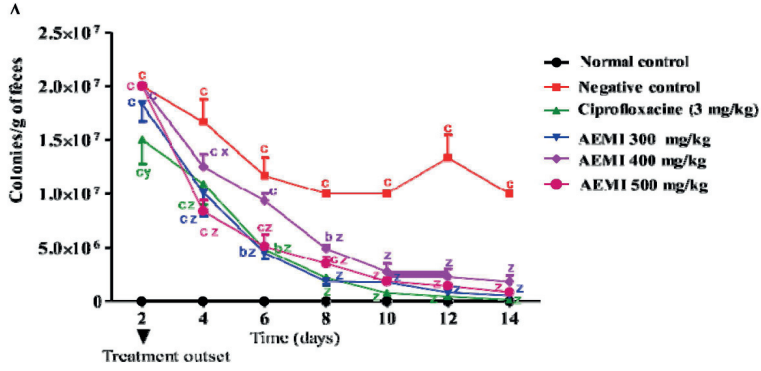
Atropine sulphate	5	360.0 ± 0.00 [c]	0.0 ± 0.0 [c]	100	0.17 ± 0.16 [c]	61.36 ± 4.00
EAMI	300	109.8 ± 7.53[c]	2.16 ± 0.17[c]	43.46	2.33 ± 0.33 [c]	80.92 ± 2.96
	400	360.0 ± 0.00 [c]	0.0 ± 0.0 [c]	100	0.33 ± 0.21[c]	20.82 ± 13.6 [c]
	500	360.0 ± 0.00 [c]	0.0 ± 0.0 [c]	100	0.68 ± 0.33 [c]	30.28 ± 15.30 [c]
EMMI	300	336.8 ± 11.86 [c]	0.66 ± 0.1[c]	82.61	1.33 ± 0.21[c]	72.21 ± 5.16
	400	354.7± 5.33 [c]	0.17 ± 0.16 [c]	95.65	0.33 ± 0.33 [c]	76.62 ± 0.0
	500	76.67 ± 6.41 [a]	1.83 ± 0.31[c]	52.8	2.83 ± 0.47 [a]	79.64 ± 3.07

Each value is expressed as mean ± SEM, (n = 6). [a]p<0.05, [b]p<0.01 and [c]p<0.001: significant differences compared to negative control.

Table 3: Effects of aqueous and methanolic stem barks extracts of *Mangifera indica* against castor oil induced motility in rats.

Treatment	Doses (mg/kg)	Total length of intestine (cm)	Traveled distance by charcoal (cm)	peristaltic index	% inhibition
Distilled water	1mL/100 g	115.3 ± 6.2	86.20 ± 3.57	0.79 ± 0.02	-
Atropine sulphate	5	105.7 ± 1.11	44.92 ± 8.13[c]	0.42 ± 0.07[c]	46.83
EAMI	300	110.6± 2.24	78.48 ± 3.12	0.70 ± 0.02	11.39
	400	111.1 ± 1.07	81.67 ± 4.31	0.72 ± 0.02	8.86
	500	114.2 ± 3.8	73.67 ± 3.19	0.68 ± 0.03	13,92
EMMI	300	109.9 ± 3.38	65.08 ± 2.49	0.58 ± 0.01[a]	26.58
	400	111.5 ± 2.23	67.83 ± 6.19	0.60 ± 0.05[a]	24.05
	500	111.0 ± 2.26	66.40 ± 4.27	0.59 ± 0.03[a]	25.31

Each value is expressed as mean ± SEM, (n = 6). [a]p<0.05 and [c]p<0.001: significant differences compared to negative control.



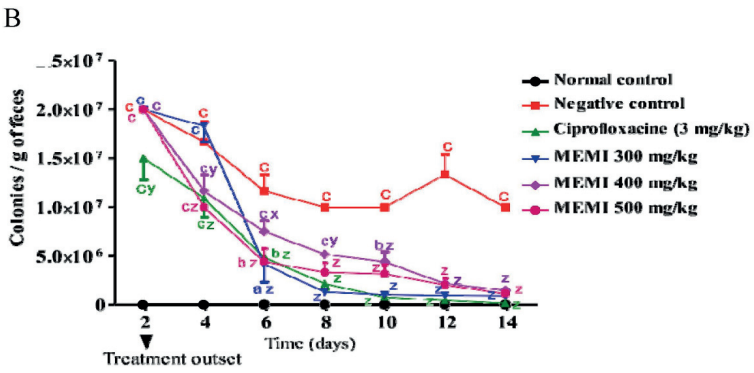


Fig. 1 Effect of aqueous (a) A and methanolic (b) extracts of M. indica on the fecal bacterial load in rats. Each value is expressed as mean ± SEM (n = 6). xp < 0.05, yp < 0.01 and zp < 0.001: significant differences compared to negative control. ap < 0.05, bp < 0.01 and cp < 0.001: significant differences compared to normal control. AEMI aqueous extract of M. indica, MEMI methanolic extract of M. indica.

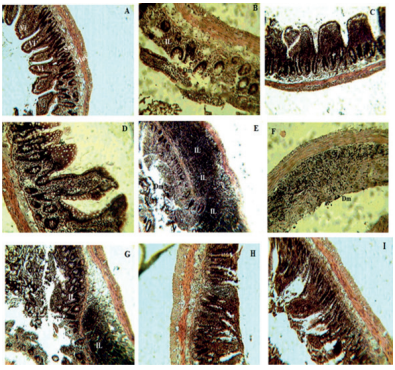


Fig. 2 Histology of ileum sections showing the effects of aqueous and methanolic stem bark extract of *M. indica* on enteropathogenic *E. coli* induced infectious diarrhoea. *Vi* villi, *Sm* submucosa, *Mus* musculeuse, *IL* infiltration leucocytes. (A = neutral control): Normal intestinal villi (Vi), submucosa and musculeuse. (B = negative control): Mucosal epithelium shedding, focal loss of the submucosa and lymphocytes infiltration (inflammation). (C = positivecontrol (ciprofloxacin 3 mg/kg)): Epithelial regeneration. (D = EAMI 300 mg/kg): Mucosal epithelial regeneration. (E = EAMI 400 mg/kg): Mucosal epithelium shedding, focal loss of the submucosa and musculeuse and lymphocytes infiltration (inflammation). (F = EAMI 500 mg/kg): Focal loss of mucosal epithelium and submucosa. (G = EMMI 300 mg/kg): Mucosal epithelium shedding, focal loss of the submucosa and musculeuse and lymphocytes infiltration (inflammation). (H = EMMI 400 mg/kg): Mucosal epithelial regeneration.(I = EMMI 500 mg/kg): Mucosal epithelial regeneration. Magnification (x200).