

## Research Article

# Antidiarrheal Activity of the Ethyl Acetate Extract of *Morinda morindoides* in Rats

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## Abstract

**Purpose:** The objective of the study was to investigate the ethyl acetate extract of *Morinda morindoides* (Baker) Milne-Redh (Rubiaceae) (MM-EA) properties against experimental diarrhea induced by castor oil in albino Wistar rats.

**Methods:** The ethyl acetate extract of *Morinda morindoides* (250, 500, and 1000 mg/kg body weight) was administered orally to three groups of rats (five animals per group) in order to evaluate the activity of the extract against castor oil-induced diarrhea model in rat. Two other groups received normal saline (5mg/kg) and loperamide (5mg/kg) as positive control. The effect of the extract on intestinal transit and castor oil-induced intestinal fluid accumulation (enteropooling) was assessed.

**Results:** At oral doses of 250, 500, and 1000 mg/kg body weight, the plant extract showed pronounced and dose-dependent antidiarrheal activity. The protective role of the extract at 1000 mg/kg was comparable to that of the reference drug, loperamide (5mg/kg). The extract (1000 mg/kg) produced a decrease in intestinal transit comparable to atropine (5mg/kg), and significantly ( $p < 0.01$ ) inhibited castor oil-induced enteropooling. No mortality and visible signs of general weakness were observed in the rats following the extract administration of up to a dose of 6000 mg/kg.

**Conclusion:** The results showed that the extract of *M. morindoides* has a significant antidiarrheal activity which supports its use in traditional herbal medicine practice.

**Keywords:** Antidiarrheal activity, Castor oil, *Morinda morindoides*, Intestinal transit, enteropooling

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## INTRODUCTION

Diarrheal diseases are a major problem in Third World countries and are responsible for the death of millions of people each year<sup>1</sup>. Diarrhoea is an alteration in normal bowel movement and is characterized by an increase in the water content, volume, or frequency of stools<sup>2</sup>. Plants have long been a very important source of new drugs. Many plant species have been screened for substances with therapeutic activity. Medicinal plants are a promising source of antidiarrheal drugs<sup>3</sup>. For this reason, international organizations including the World Health Organization (WHO) have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices<sup>4,5,6</sup>. *Morinda morindoides* (Baker) Milne-Redh (Rubiaceae) is found in the borders of tropical forests. In the Democratic Republic of Congo, *M. morindoides* has long been used in villages and towns in the treatment of some parasitic diseases, and the leaf extracts of the plant have been shown to possess antiprotozoal activity particularly against *Entamoeba histolytica* and rheumatic pain<sup>7,8</sup>. The decoction of the leaves is used for the treatment of malaria, intestinal worms, and amoebiasis<sup>9,10</sup>. Recently Zirihi *et al*<sup>11</sup> have shown that the ethanol extract of *M. morindoides* exhibited good *in vitro* antimalarial activity against chloroquine-resistant FcB1/Colombia strain of *Plasmodium falciparum*.

Ten flavonoids which were identified as quercetin, quercetin-7, 4-dimethylether, quercetin-3-*O*-rutinoside, quercetin-3-*O*-rhamnoside, kaempferol-3-*O*-rhamnoside, kaempferol-3-*O*-rutinoside, kaempferol-7-*O*-rhamnosylsophorose, chrysoeriol-7-*O*-neohesperidoside, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside<sup>12-14</sup> have been isolated from the butanol and ethyl acetate fractions of the plant leaves. In addition, a total of eight iridoid glycosides, among which are gaertneroside and gaertneric acid, methoxygaertneroside and

epoxygaertneroside, were also isolated from the same fractions by Cimanga *et al*<sup>6</sup>.

*M. morindoides* is well known in the traditional medical practice of the west central part of Ivory Coast. It is commonly called Zélékelé in the local language of 'Bété' and is used as an antifungal agent. The leaves of the plant are used in traditional medicine to treat diarrhoea. The present work was undertaken to investigate the potential *in vivo* antidiarrheal effect of the ethyl acetate extract of *M. morindoides* in various experimental models of diarrhoea in rats.

## MATERIALS AND METHODS

### *Plant material*

The leaves of *Morinda morindoides* (Rubiaceae) were collected from Daloa (central west region of Ivory Coast) in June 2006. The plant was identified and authenticated by Pr AKE ASSI, of the Department of Botany, University of Cocody. A voucher specimen (no17710) of the plant was deposited in the herbarium of the National Floristique Center of the University of Cocody-Abidjan.

### *Preparation of ethyl acetate extract*

The leaves of *M. morindoides* were cleaned of extraneous matter, air-dried at room temperature for 7 days and ground into a fine powder. The powder was mixed with distilled water (80 g of powder in 2 L of distilled water) for 24 h with constant stirring at 80°C. The extract was filtered twice through cotton wool, then through Whatman filter paper (No. 1). The filtrate was evaporated to dryness using a rotary evaporator (Buchi). Twenty five grams (25 g) of the dry aqueous extract was dissolved in 500 ml of ethanol and water (consisting of 350 ml of ethanol 96% and 150 ml of distilled water) and after thorough mixing, the supernatant was evaporated using a rotary evaporator. Following the method of Guede-Guina *et al*<sup>15</sup>, 25 g of the dried extract was dispersed in 500 ml of a

solution (made up of 250 ml of ethyl acetate and 250 ml of distilled water), and mixed for 24 h with constant stirring. From the two phases formed, the supernatant was evaporated using a rotary evaporator, and the resulting dry powder was taken as the ethyl acetate extract.

### **Animal**

Albino Wistar rats (weighing 150 - 200 g) of both sexes, were housed in standard metal cages. They were provided with food and water *ad libitum*, and allowed a one-week acclimatization period prior to the study. The equipment, handling and sacrificing of the animals were in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals<sup>16</sup>.

### **Drug and chemicals**

Atropine sulphate and loperamide (Sigma Chemical Co, St Louis, Mo, USA), castor oil (Qualikems Fine Chemicals Pvt. Ltd, New Delhi, India), normal saline (NaCl 0.9%) and charcoal meal (10% activated charcoal in 100 ml of 5% aqueous gum acacia) were used.

### **Preliminary acute toxicity test**

The extract of *M. morindoides* was administered orally in doses of 125, 250, 500, 1000, 2000, 4000 and 6000 mg/kg body weight to animal groups (one dose per group). Simultaneously, the control animals received normal saline (5ml/kg). The general signs and symptoms of toxicity, intake of food and water and mortality were recorded for a period of 48 h and then for a period of 14 days.

### **Castor oil-induced diarrhea in rats**

Twenty five (25) rats were fasted for 18 h and divided into five groups of five animals each. The plant extract (250, 500, and 1000 mg/kg body weight) were administered orally to groups 1, 2 and 3, respectively. The fourth group received normal saline (5 ml/kg body

weight) and served as control, while the fifth group received the standard drug, loperamide (5 mg/kg body weight). One hour later, all the animals received 2 ml/rat of castor oil orally by gavage. The animals were kept in separate metabolic cages with a transparent plastic container beneath the cage to collect faeces<sup>17</sup>. The severity of diarrhoea was assessed each hour for 6 h. The total number of faeces (both diarrheal and non-diarrheal) expelled were compared with the control group. The total score of diarrheal faeces for the control group was considered as 100%. The results were expressed as a percentage of inhibition of diarrhoea.

### **Gastrointestinal motility test**

The rats were divided into five groups of five animals each and fasted for 18 h but water was freely provided. The first group (control group) received orally normal saline (5 ml/kg body weight), while the second, third and fourth groups were given orally the plant extract in doses of 250, 500, and 1000 mg/kg body weight, respectively. The fifth group received orally the standard drug, atropine sulphate (5 mg/kg body weight). Thirty (30) min later, each animal was given 1 ml/rat of charcoal meal (10% activated charcoal in 5% gum acacia) via the oral route. All animals were sacrificed 30 min thereafter, and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as percentage of distance moved<sup>18</sup>.

### **Castor oil-induced enteropooling**

Intraluminal fluid accumulation was determined by the method of Robert *et al*<sup>19</sup>. Rats were divided into five groups of five animals each, one hour before oral administration of castor oil (2 ml/rat). Group 1 received normal saline orally (5 ml/kg body weight), and served as the control. Group 2 animals received loperamide (5mg/kg, oral) while groups 3, 4, and 5 received, by oral intubation, the extract of *M. morindoides* at doses of 250, 500 and 1000 mg/kg body

weight, respectively. Two hours later, the rats were sacrificed and the small intestine from the pylorus to the caecum was isolated. The intestinal contents were collected by milking into a graduated tube and their volume measured.

### Statistical analysis

Data were analyzed by one-way ANOVA followed by Dennett's t-test using InStat® (Graph Pad software, U.S.A). At 95% confidence interval  $p < 0.05$  was considered statistically significant.

## RESULTS

### Preliminary acute toxicity test

It was observed that oral administration of the extract of *M. morindoides* to the rat up to 6000 mg/kg neither showed mortality nor any apparent signs of weakness in the animals.

### Effect of *M. morindoides* extract on castor oil-induced diarrhoea

In the castor oil-induced diarrhoea experiment, the extract of *M. morindoides* produced a marked antidiarrheal effect in the rats, as shown in Table 1. At doses of 250, 500, and 1000 mg/kg, the extract significantly decreased ( $p < 0.01$ ) the total number of wet faeces produced upon administration of castor oil ( $12.20 \pm 1.06$  at 250 mg/kg,  $7.00 \pm 0.94$  at 500 mg/kg and  $6.20 \pm 0.58$  at 1000 mg/kg) compared to the control group ( $18.6 \pm 0.74$ ). The effect of the highest dose of the extract was similar to that of the standard drug, loperamide (5 mg/kg).

### Effect of *M. morindoides* extract on intestinal transit of charcoal meal

The extract of *M. morindoides* decreased propulsion of charcoal meal in the rat gastrointestinal tract at oral doses of 250 - 1000 mg/kg, compared with the control group that receiving normal saline (5 mg/kg). A similar reduction in the gastrointestinal transit

of charcoal meal in rat was achieved with atropine sulphate (5 mg/kg). The results are shown in Table 2.

### Effect of extract of *M. morindoides* on castor oil-induced enteropooling

*Morinda morindoides* extract significantly ( $P < 0.01$ ) inhibited castor oil-induced enteropooling in rats at oral doses of 500 and 1000 mg/kg (Table 3). The intestinal fluid in control animals was  $3.44 \pm 0.36$  ml. The inhibition of intestinal accumulation was 24.41% ( $P < 0.01$ ), 52.32% ( $P < 0.01$ ) and 61.04 % ( $P < 0.01$ ) at doses of 250, 500, and 1000 mg/kg of the extract, respectively. The standard drug, loperamide (5 mg/kg), also significantly inhibited ( $P < 0.01$ ) intestinal fluid accumulation (59.30%).

## DISCUSSION

In the traditional medicine system, *Morinda morindoides* is used in the management of diarrhoea by traditional medicine practitioners in Ivory Coast. The present study sought to assess the antidiarrheal activity of the plant.

Our results showed that the extract inhibited significantly ( $p < 0.01$ ) castor oil-induced diarrhoea in rats. Several mechanisms had been previously proposed to explain the diarrheal effect of castor oil. These include inhibition of intestinal  $\text{Na}^+ \text{K}^+$  ATPase activity, thus reducing normal fluid absorption<sup>20</sup>, activation of adenylate cyclase or mucosal cAMP-mediated active secretion<sup>21</sup>, stimulation of prostaglandin formation<sup>22</sup>, and platelet activating factor<sup>23</sup>. Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil<sup>24</sup>. However, it is well documented that castor oil produces diarrhoea due to its most active component ricinoleic acid through a hypersecretory response<sup>25,26</sup>. Therefore it can be assumed that the antidiarrheal action of the extract was mediated by an antisecretory mechanism. This was also evident from the inhibition of castor oil-induced fluid accumulation by the extract. The results were comparable to those of the standard drug, loperamide.

**Table 1:** Effect of the extract of *M. morindoides* (MM-EA) on castor-oil induced diarrhoea in rat. Values are expressed as mean  $\pm$  S.E.M (n = 5). \*\*P<0.01, when compared to the control.

Treatment	Total number of faeces	Number of diarrhoea faeces	Inhibition of diarrhoea (%)
Normal saline (5 mg/kg) +Castor oil (2 ml)	22.80 $\pm$ 0.86	18.6 $\pm$ 0.74	
Loperamide (5 mg/kg) + Castor oil (2 ml)	9.80 $\pm$ 1.65**	5.20 $\pm$ 1.15**	72.04
MM-EA (250 mg/kg)+ Castor oil (2 ml)	19.80 $\pm$ 0.73	12.20 $\pm$ 1.06**	34.40
MM-EA (500 mg/kg) + Castor oil (2 ml)	16.40 $\pm$ 1.12**	7.00 $\pm$ 0.94**	62.36
MM-EA (1000 mg/kg) + Castor oil (2 ml)	15.60 $\pm$ 1.80**	6.20 $\pm$ 0.58**	67.00

Values are expressed as mean  $\pm$  S.E.M (n = 5) \*\*p < 0.0 1, when compared to the control

**Table 2:** Effect of extract of *M. morindoides* (MM-EA) on the intestinal transit of charcoal meal in rat

Treatment	Distance travelled by charcoal (%)	Inhibition (%)
Normal saline (5 mg/kg)	73.95 $\pm$ 3.55	
Atropine (5 mg/kg)	30.85 $\pm$ 1.69**	58.28
MM-EA (250 mg/kg)	61.81 $\pm$ 3.45*	16.41
MM-EA (500 mg/kg)	48.00 $\pm$ 2.59**	35.09
MM-EA (1000 mg/kg)	35.62 $\pm$ 3.63**	51.83

Values are expressed as mean  $\pm$  S.E.M (n = 5) \*P < 0.05, \*\*p < 0.0 1, when compared to the control

**Table 3:** Effect of extract of *M. morindoides* (MM-EA) on castor-oil induced enteropooling in rat

Treatment	Volume of intestinal fluid (ml)	Inhibition (%)
Normal saline (5 mg/kg) + Castor oil (2 ml)	3.44 $\pm$ 0.36	
Loperamide (5 mg/kg) + Castor oil (2 ml)	1.40 $\pm$ 0.24**	59.30
MM-EA (250 mg/kg) + Castor oil (2 ml)	2.60 $\pm$ 0.55	24.41
MM-EA (500 mg/kg) + Castor oil (2 ml)	1.64 $\pm$ 0.20**	52.32
MM-EA (1000 mg/kg) + Castor oil (2 ml)	1.34 $\pm$ 0.09**	61.04

Values are expressed as mean  $\pm$  S.E.M (n = 5). \*\*P<0.01, when compared to the control

Furthermore, the extract significantly reduced intestinal transit as evidenced by the decrease in the distance traveled by charcoal meal. These results also show that the extract suppressed the propulsion of charcoal meal thereby increasing the absorption of water and electrolytes. Antidiarrheal properties of medicinal plants were found to be due to

tannins, flavonoids, alkaloids, saponins, reducing sugar, sterols and/or terpenes<sup>27,28</sup>. The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions<sup>29,30</sup> which are altered in this intestinal condition. *In vitro* and *in vivo* experiments have shown that flavonoids are

able to inhibit the intestinal secretory response induced by prostaglandins  $E_2$ <sup>31</sup>. In addition, flavonoids present antioxidant properties<sup>32</sup> which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism<sup>33</sup>. These constituents may be responsible for the antidiarrheal activity of the ethyl acetate extract of *M. morindoides*.

## CONCLUSION

The results of this investigation revealed that *M. morindoides* contains pharmacologically active substances with antidiarrheal properties. These attributes may provide the rationale for the use of *Morinda morindoides* in diarrhoea management by traditional healers. Further research is needed to fractionate the ethyl acetate extract and isolate the molecule(s) responsible for the antidiarrheal activity observed.

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