



Effect of Aqueous Ethanol Stem Extract of *Entada africana* Guill. Et Perrott. on Castor Oil and Magnesium Sulphate-Induced Diarrhoea Models in Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Author ABM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MIU managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigated the phytochemical, elemental analysis, acute toxicity study of aqueous ethanol stem bark extract of *Entada africana*, as well as its antidiarrhoeal activity in mice and its effects on isolated rabbit jejunum. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, cardiac glycosides, phenols, saponins, steroids, tannins and terpenoids. Elemental analysis of the extract showed the presence of magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), lead (Pb), zinc (Zn) and sodium (Na) while acute toxicity study revealed intraperitoneal median lethal dose (LD₅₀) values for the extract to be 774.6 mg/kg body weight. The antidiarrheal effect of the extract was studied using castor-oil and magnesium sulphate induced diarrhoeal models (dropping test) and gastrointestinal transit test in mice. The result showed that the extract produced a dose-dependent protection against diarrhoea induced by castor oil and

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magnesium sulfate, with the highest protection (80 and 100%), obtained at 100 and 200 mg/kg. The extract significantly ($p \leq 0.01$) reduced the small intestinal transit of charcoal meal in mice at all doses tested. The extract (0.4-3.2 mg/ml) produced a concentration dependent relaxation of the rabbit jejunum, and the effects were blocked by propranolol (0.04 and 0.64 $\mu\text{g/ml}$). The results of this study showed that the extract contain pharmacologically active substance with antidiarrhoeal properties mediated through inhibition of hyper secretion and reduced gastrointestinal motility. These properties may explain the rationale for use of it's stem bark as antidiarrhoeal remedy in traditional medicine.

Keywords: Antidiarrhoeal activity; castor oil; *Entada africana*; stem bark extract and magnesium sulphate.

1. INTRODUCTION

Diarrhoea is the passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual [1]. It may be a symptom of gastrointestinal infection, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection can be spread through contaminated food or drinking-water, or from person to person as a result of poor hygiene [2]. Diarrhoea is the most common clinical manifestation of gastrointestinal disease which can be caused by both infectious and non-infectious agents. The onset of diarrhoea may be abrupt and self-limiting in immune-competent individuals. Dehydration which also occurs as a result of diarrhoea is a condition of hypertonic hypovolemia brought about by the net loss of hypotonic body fluids, severe dehydration is a medical emergency and can be life-threatening, death may follow if dehydration is not started quickly [2].

Drugs which are used in treatment of diarrhoea have also been found to cause side effects like nausea and constipation amongst many with most causing depression of the immune system. Thus, there is need for search of drugs that offer less of these toxicities.

Entada africana Guill. Et Perrott. (Fam: Mimosaceae), commonly known as 'Adans' is a small tree up to 4-10 m in height and 90 cm in girth; branching low down, with a wide crown; bark brown-grey to black, very rough, transversely striped, scaly, peeling in long fibrous strips, slash fibrous, red or yellow-brown [3]. The leaves of *Entada africana* make good fodder and its stem-bark fiber are used for ropes and bands. The bark of the root and stem yields a long fiber used for cordage, commonly for roof binding and grass matting. The wood is light red, soft and easy to work with [4]. *Entada africana* have shown promising potential against analgesia,

inflammation, and heme biomineralization inhibitory property [5], it was also reported to have antimicrobial, antiparasitodal, haemolytic and antioxidant activity [5]. The infusion of roots is used as an eye lotion in Zambia [3]. The plant is used for the treatment of diabetes, hypertension and diarrhoea in Burkina Faso.

This study investigated the antidiarrhoeal activity of aqueous ethanol stem bark extract of *Entada africana* in castor oil, magnesium sulphate-induced diarrhoea and gastrointestinal transit models in mice.

2. MATERIALS AND METHODS

2.1 Chemicals and Equipment

All reagents and solvents used in this experiment were of analytical grade. The chemicals used include: Activated Charcoal (Merck KGaA, Darmstadt, Germany), Loperamide Hydrochloride (Jiangsu Ruinian Qianjin Pharmaceutical Co., Ltd.), Castor oil (Bell Sons and Co., England), Acetylcholine (Sigma Chemicals, USA), Magnesium Sulphate (BDH Chemicals Ltd. Poole, England), Propranolol (AstraZeneca UK Ltd.), Isoprenaline (Sigma Chemicals, USA), Acacia Powdered (BP Evans Medical Ltd.), 0.1% Tween 80, tyrode solution (composition in mM- NaCl, 137; CaCl₂, 1.8; KCl, 2.7; glucose, 5.55; NaHCO₃, 11.9; MgCl₂, 1; NaH₂PO₄, 0.4). Microdynamometer 7050 (Ugo Basile), Mettler P162 analytical balance.

2.2 Plant Collection and Identification

The fresh stem bark of *Entada africana* was collected from Gwaram Local Government Area of Jigawa State, Nigeria. It was identified and authenticated at the Herbarium Section, Department of Plant Biology, Faculty of Sciences, Bayero University Kano, by comparing with already deposited voucher specimen No. 0192.

The stem bark was air-dried and crushed into coarse powder using pestle and mortar. The powder stem bark was soaked in 70% v/v ethanol (in water) for 14 days with occasional shaking. The resultant mixtures were filtered using Whatman filter paper (No. 1). The filtrate was collected into a porcelain dish and concentrated to dryness using water bath maintained at 60°C. The dried extract was weighed and the percentage yield calculated. The resultant extract was stored in an airtight container for use.

2.3 Experimental Animals

New Zealand Rabbit weighing 1.6 kg and Swiss mice 20–25 g maintained in the Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria were used for the experiments. The animals were housed in steel cage under standard conditions and fed with standard laboratory feeds (Vital Feed Nig. LTD) and water provided *ad libitum*.

2.4 Phytochemical Screening

Preliminary phytochemical screening was carried out on aqueous ethanol stem bark extract of *Entada africana* using standard protocol [6].

2.5 Elemental Analysis

The crude powder (2 g) of aqueous ethanol stem bark extract of *Entada africana* was ashed in an oven at 60°C for three hours, about 0.5 g of the sample was then acid digested by addition of 10 ml Hydrochloric acid (HCl), Nitric acid (HNO₃) and perchloric acid (HClO₄) respectively. The flask was then heated in an electro thermal heater with gentle swirling till digestion is completed by evolution of white fumes. The digested mixture was evaporated down to 5 ml using rotatory evaporator; it was then made up to 10 ml with 2M HNO₃, and to which were added 30 ml of distilled water and kept in a 100 ml beaker. The resulting solution was used for the elemental analysis using atomic absorption spectrophotometer [7].

2.6 Acute Toxicity Study

The median lethal dose (LD₅₀) of the extract was determined intraperitoneally in mice using the method described by Lorke [8]. It was carried out in two phases, in the first phase, three (3) groups of three (3) mice each were administered with

the extract at doses of 10, 100 and 1000 mg/kg body weight intraperitoneally respectively. The mice were then monitored for 24 hours for signs and symptoms of toxicity, and mortality. In the second phase, four (4) groups of one (1) mouse each were further administered with doses of 140, 225, 370 and 600 mg/kg body weight. The mice were also observed for 24 hours, for signs and symptoms of toxicity including mortality. The LD₅₀ values were then calculated as the geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred) of which there is 1/1 and 0/1.

2.7 Anti-Diarrhoea Studies

2.7.1 Castor oil-induced diarrhoea in mice

According to the method of Awouters et al. [9] after an overnight fast, the mice were randomly divided into 5 groups of 5 mice each. Group I was administered normal saline in 0.1% Tween 80 orally. The mice in Groups II, III and IV were pretreated with 200, 100 and 50 mg/kg of aqueous ethanol stem bark extract of *Entada africana* (*i.p*) and group V were administered loperamide at 5 mg/kg (*i.p*). After 30 minutes castor oil 0.2 ml/mouse was administered intragastrically. The mice were then placed on individual cages over clean filter paper. Three hours after the administration of the castor oil, the cages were inspected for the presence of characteristic diarrhoea. Their absence was recorded as protection from diarrhoea, and the percentage protection was calculated.

2.7.2 Magnesium sulphate induced diarrhoea in mice

According to the method of [9]. After an overnight fast, the mice were randomly divided into 5 groups of 5 mice each. Group I was administered normal saline in 0.1% Tween 80 orally. The mice in Groups II, III and IV were pretreated with 200, 100 and 50 mg/kg of aqueous ethanol stem bark extract of *Entada africana* (*i.p*) and group V were administered loperamide at 5 mg/kg (*i.p*). After 30 minutes 2 g/Kg magnesium sulphate was administered intragastrically to each mouse according to their body weight. The animals were then placed on individual cages over clean filter paper. Three hours after the administration of the magnesium sulphate, the cages were inspected for the presence of characteristic diarrhoea. Their absence was recorded as protection from diarrhoea, and the percentage protection was calculated.

2.7.3 Gastrointestinal motility (Charcoal meal transit)

According to the method described by Jabbar et al. [10] was used. After an overnight fast, the mice were randomly divided into 5 groups of 5 mice each. Group I was administered normal saline in 0.1% Tween 80 orally. The animals in Groups II, III and IV were pretreated with 200, 100 and 50 mg/kg of aqueous ethanol stem bark extract of *Entada africana* (*i.p*) and group V was administered loperamide at 5 mg/kg (*i.p*). Five minutes after administration of normal saline, extract and loperamide, 0.5 ml of 10% charcoal in 5% gum acacia was administered to each mouse intragastrically. All mice were sacrificed by cervical dislocation 30 minutes later, the abdomen was open and the total length of the small intestine was measured with a calibrated ruler. The distanced travelled by the charcoal plug from the pylorus to the caecum was determined and expressed as percentage of the total length of the intestine from where the percent inhibition of movement was calculated by subtracting the percentage travelled from 100%.

2.7.4 Isolated tissue studies

According to the method described by Amos et al. [11], overnight starved rabbits (weighing 2 kg) were sacrificed. The abdomen was opened and a piece of ileum was dissected and placed in oxygenated Tyrode's solution at room temperature. Longitudinal strips of ileum 2 – 3 cm long were then prepared and mounted under a 2 g tension in a 25 mL organ bath filled with Tyrode's solution at 37°C and maintained under constant aeration with carbogen gas (95% O₂ + 5% CO₂). The tissue was washed several times with fresh Tyrode's solution and allowed to rest and equilibrate for 30 min to achieve a stable basal rhythm. The contact time for each concentration was 1 min and the tissues were washed three times before new administration; the responses were recorded using a microdynamometer with speed of 24 mm/min and a sensitivity of 2.

2.8 Statistical Analysis

The data obtained were expressed as Mean ± SEM (standard error of mean) or percentage protection. Data were analyzed using one way analysis of variance (ANOVA), followed by Dunnett's t-test to find out the level of significance. Values of P ≤ 0.05 were considered statistically significant.

3. RESULTS

3.1 Phytochemical Screening

The phytochemical screening of ethanol stem bark extract of *Entada africana* revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids, tannins and terpenoids, while anthraquinone and volatile oil were absent (Table 1).

Table 1. Phytochemical constituents of aqueous ethanol stem bark extract of *Entada africana*

Constituents	Inference
Alkaloids	+
Anthraquinone	-
Carbohydrates	+
Flavonoids	+
Cardiac Glycosides	+
Phenols	+
Proteins	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+
Volatile oil	-

Key: + = (Positive) present and - = (Negative) absent

3.2 Elemental Analysis

The elemental analysis of *Entada africana* ethanol stem bark extract showed the presence of magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), lead (Pb), zinc (Zn) and sodium (Na) at different concentrations (Table 2).

Table 2. Elemental constituents of aqueous ethanol stem bark extract of *Entada africana*

Elements	Concentration µg/L
Copper	2.3
Iron	18.8
Lead	42.7
Magnesium	1010.1
Manganese	12.9
Sodium	989.4
Zinc	6.4

3.3 Acute Toxicity Study

The median lethal dose of aqueous ethanol stem bark extract of *Entada africana* was determined as 774.6 mg/kg.

3.4 Castor-Oil Induced Diarrhoea

Aqueous ethanol stem bark extract produce a dose dependent increase in protection when compared with normal saline administered group. The extract at doses of 50, 100 and 200 mg/kg produced 60, 80, and 100% protection respectively while the standard drug (Loperamide) at a dose of 5 mg/kg produce 100% protection from diarrhoea (Table 3).

3.5 Magnesium Sulphate – Induced Diarrhoea

Aqueous ethanol stem bark extract produce a dose dependent increase in protection when compared with normal saline administered group. The extract at doses of 50, 100 and 200 mg/kg produced 40, 80 and 100% protection respectively. Loperamide a standard drug at a dose of 5 mg/kg produce 100% protection from diarrhoea (Table 4).

3.6 Gastrointestinal Motility (Charcoal Meal Transit)

Aqueous ethanol stem bark extract produce a significant ($p \leq 0.01$) inhibition of charcoal movement at doses of 50, 100 and 200 mg/kg in mice, when compared to normal saline treated group. However, the inhibition is not significant when compared to loperamide administered group at dose of 5 mg/kg (Table 5).

3.7 Effect Aqueous Ethanol Stem Bark Extract of *Entada africana*, Isoprenaline and Propranolol on Isolated Rabbit Jejunum

The extract induced a concentrations dependent (0.4-3.2 mg/ml) relaxation of rabbit jejunum (Table 6a) similar to a standard drug Isoprenaline a non-selective β -agonist also at concentration of 0.008 μ g/ml. the relaxant effect of both the

Table 3. Effect of aqueous ethanol stem bark extracts of *Entada africana* on castor oil induced diarrhoea in mice

Treatment	Dose (i.p) mg/kg	Quantal protection	Protection (%)
Normal Saline(mL/kg)	10.00	0/5	0
Extract	50.00	3/5	60
	100.00	4/5	80
	200.00	5/5	100
	Loperamide	5.00	5/5

n=5

Table 4. Effect of aqueous ethanol stem bark extract of *Entada africana* on magnesium sulphate- induced diarrhoea in mice

Treatment	Dose (i.p) mg/kg	Quantal protection	Protection (%)
Normal Saline(mL/kg)	10.00	0/5	0
Extract	50.00	2/5	40
	100.00	4/5	80
	200.00	5/5	100
	Loperamide	5.00	5/5

n=5

Table 5. Effect of aqueous ethanol stem bark extract of *Entada africana* on gastrointestinal motility (Charcoal Meal Transit) in mice

Treatments mg/kg	Dose (i.p)	Mean distance travel by the charcoal (cm)	Mean distance travel by the charcoal (%)
Normal Saline(mL/kg)	10.00	33.50 \pm 1.79	86.7
Extract	50.00	7.60 \pm 1.18 a	18.6
	100.00	6.70 \pm 2.28 a	15.7
	200.00	6.12 \pm 1.17 a	15.2
	Loperamide	5.00	4.74 \pm 2.47

Mean Distance travel by the Charcoal (cm) are expressed as mean \pm SEM; (*n*=5); a = $p \leq 0.01$ when compared with Normal saline using one way Anova followed by Dunnett's *t*-tests

Table 6a. Effect of aqueous ethanol stem bark extract *Entada africana* on rabbit jejunum

Organ bath concentration (mg/ml)	Basal contraction (cm)	Response (cm)
0.4	3.48 ± 0.04	1.34 ± 0.07
0.8	2.04 ± 0.05	1.54 ± 0.04
1.6	2.04 ± 0.02	1.74 ± 0.02
3.2	1.98 ± 0.04	1.94 ± 0.05

Values are expressed as Mean ± SEM, n=5

Table 6b. Effect of propranolol on isoprenaline and aqueous ethanol stem bark extract on Rabbit Jejunum

Organ bath concentration (mg/ml)	Basal contraction (cm)	Response (cm)
Extract 1.6	2.47 ± 0.20	1.13 ± 0.38
Isoprenaline 0.008	2.63 ± 0.30	1.00 ± 0.06
Propranolol + Isoprenaline (0.04) (0.008)	1.40 ± 0.06	0.10 ± 0.06
Propranolol + Extract (0.04) (1.6)	0.97 ± 0.12	0.03 ± 0.03

Values are expressed as Mean ± SEM, n=3

extract and Isoprenaline were blocked by propranolol a non-selective β -antagonist at concentration 0.04 μ g/ml (Table 6b).

4. DISCUSSION

Preliminary phytochemical screening of *Entada africana* ethanol stem bark extract revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, steroids, tannins and terpenoids, their presence were also reported by Tibiri et al. [12] in the organ extract; Bako et al. (2005) in barks and leaves; Gidado et al. (2013) and Njyou et al. (2013) in the stem bark extract [4] also reported the presence of alkaloids, cardiac glycosides, proteins, steroids, tannins and terpenoids in the stem-bark extract. However, in contrast to the study of Tibiri et al. [12] the extract showed the presence of alkaloids and cardiac glycosides. This difference may be explained by the fact that variation may sometimes occur in bioactive compounds of the different part of the same plant and even in the same plant parts found in different environment [13].

The elemental analysis of the extract showed the presence of macro and micro nutrients, the level of elements in plants depends on environmental conditions, such as type of soil, rainfall, vicinity of industry and extensive agricultural activity [14]. The elemental analysis showed the presence of zinc, which is an important element responsible for many enzymatic processes [14]. Normal levels of zinc can prevent and treat diarrhoea in children because of it essential micronutrient for

protein synthesis, cell growth and differentiation, immune function, and intestinal transport of water and electrolytes [15]. Zinc is also important for normal growth and development of children both with and without diarrhoea [16]. The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) in 2004 [17] recommend the use of zinc for the treatment of diarrhoea, because it reduces the duration and severity of diarrhoeal episodes which may prevent future episodes for up to three months.

Intraperitoneal median lethal dose (LD₅₀) determination of the extract in mice showed that the extract is moderately toxic (774.6 mg/kg) according to LD₅₀ classification [8].

Diarrhoea occurs as a result of an imbalance between the absorptive and secretory mechanisms in the intestinal tract which is accompanied by hypermotility, resulting in an excess loss of fluid in the faeces. The secretory component predominates in some diarrhoea, while others are characterized by hypermotility. The protection against castor oil induced diarrhoea showed by the extract may be due to ability of the extract to act on presynaptic μ -receptor located on cholinergic nerve terminal of gut, thereby inhibiting the gut motility as well as reducing electrolyte and water secretion [18]. Also the extract showed protection against magnesium sulphate induced diarrhoea by counteracting the increased osmotic imbalance caused by magnesium sulphate due to increase in electrolyte secretion [19].

The suppressed intestinal propulsive movement of the charcoal meal by the extract suggests antidiarrhoeal activity of the plant extract. This may be due to the ability of the extract to increase the time for absorption of water and electrolytes in the manner similar to the action of loperamide. Delay in gastric motility causes further absorption of water from faeces and may additionally contribute to reducing its watery texture.

On the isolated tissue, the effect of the stem bark extract on rabbit jejunum in the presence of antagonist was determined in order to find its possible mechanism of action. Results from this study showed that the extract exhibited a concentration-dependent relaxant activity on rabbit jejunum similar to that produced by standard drug isoprenaline, a non-selective beta receptor agonist. The relaxant effects of the extract and Isoprenaline were blocked by propranolol, a non-selective beta receptor antagonist, suggesting that the extract may be acting via beta adrenergic receptors. There are two possibilities for the mechanism by which the relaxant effect occur, either through adrenergic pathway or direct smooth muscle relaxant effects. Flavonoids have been known to inhibit contractions induced by spasmogenes [20]. The presence of flavonoids in the plant extract might be responsible for the observed concentration-dependent relaxation of the rabbit jejunum. The relaxant effect also explains why these extract could protect the mice against diarrhoea induced by castor oil and magnesium sulphate, a stimulant laxative.

Antidiarrhoeal properties of medicinal plants can be attributed to their phytochemical constituents; studies have related antidiarrhoeal properties to the presence of tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes [21]. Flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) [22,23]. Certain flavonoids inhibit inflammatory processes by inhibiting key enzymes involved in the synthesis of prostaglandins [24]. Flavonoids were reported to possess antidiarrhoeal activity which is attributed to their ability to inhibit intestinal motility and hydro-electrolytic secretion [25]. Flavonoids and terpenoids derivatives are known for inhibiting release of autacoids and prostaglandins, thereby inhibiting the motility and secretion induced by castor oil [26].

Tannins were reported to denature proteins in the intestinal mucosa by forming protein tannates

which may reduce secretion. Studies on the functional role of tannins also revealed that they produce similar functions by reducing the intracellular Ca^{2+} [27]. The antidiarrhoeal activity of the stem bark extract may be due to the presence of flavonoids singly or in combination with other constituents present, these constituents may be responsible for the in vivo antidiarrhoeal activity of the plant.

5. CONCLUSION

The study showed that administration of the aqueous ethanol stem bark extract of *Entada africana* at 200 mg/kg protect the mice against diarrhoea induced by castor oil and magnesium sulfate, as loperamide a standard drug. The extract also produced a significant ($p < 0.01$) and dose-dependent delay in charcoal transit in mice. Thus, the possible mechanism for antidiarrhoeal activity of the extract could be via adrenergic receptors attributed to flavonoids and other phenolic compounds present in the extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Principle of laboratory animal care and ethical guidelines for animals were observed during experimentation (NIH, 1996; Zimmermann, 1986).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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