Original Research Article

EFFECT OF ETHANOL STEM EXTRACT OF *ENTADA AFRICANA* GUILL. ET PERROTT. ON CASTOR OIL AND MAGNESIUM SULPHATE-INDUCED DIARRHOEA MODELS IN MICE

ABSTRACT

This study was aimed at investigating the *Entada africana* ethanol stem bark extract on phytochemical, elemental analysis, acute toxicity study, antidiarrheal 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 (LD_{50}) 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 (charcoal transit) in mice. The result showed the extract produced a dose-dependent protection against diarrhoea induced by castor oil and 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 where blocked by propranolol (0.04 and 0.64µg/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 the plant as antidiarrheal agent in traditional medicine.

Keywords: ***Antidiarrhoeal activity; Castor Oil; Entada Africana; stem bark extract and Magnesium Sulphate.***

1.0 INTRODUCTION

Diarrhoea is the passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual. It is usually a symptom of gastrointestinal infection, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person to person as a result of poor hygiene (WHO, 2013). 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 (Njume and Goduka, 2012). 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 from dehydration can occur in three days or less.

Drugs which are being 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 (Gralla *et al.,* 2005). 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 4-10m in height and 90cm 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 (Keay, 1989; Orwa *et al.,* 2009). 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 (Mbatchou *et al*., 2011). *Entada africana* shown promising potential against analgesia, inflammation, and heme biomineralization inhibitory property (Ezenyi *et al.,* 2014), it was also reported to have antimicrobial, antiplasmodial, haemolytic and antioxidant activity (Karou *et al*., 2011; Ezenyi *et al.,* 2014). The infusion of roots is used as an eye lotion in Zambia. (Orwa *et*

al., 2009). The plant is used for the treatment of diabetes, hypertension and diarrhoea in Burkina Faso (Nacoulma-Ouedraogo, 1996).

In order to search for newer remedy for diarrhoea, this study aimed at the investigation of the antidiarrhoeal activity of ethanol stem bark extract of *Entada africana* in castor oil, magnesium sulphate-induced diarrhoea and gastrointestinal transit models in mice.

2.0 MATERIALS AND METHODS

2.1 Collection and extraction of Plant Materials

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. It was cleaned, air-dried and size reduced into coarse powder using pestle and mortar. About 238 g of the coarse leaves powder were cold macerated separately in 3 of 70% $^{v}/_{v}$ ethanol (in water) for 14 days with occasional shaking. The resultant mixtures were filtered using Whatman filter paper (No. 1) and concentrated to dryness using water bath maintained at 60^{o}C. The extract was then stored in desiccators. Solution of the extract was prepared freshly for each study.

2.2 Experimental Animals

New Zealand Rabbit weighing 1.6 kg and Swiss albino mice 20–25g 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 and water provided *ad libitum*. Principle of laboratory animal care and ethical guidelines for investigation of experimental pain in conscious animals were observed during experimentation (NIH, 1996; Zimmermann, 1986).

2.3 Phytochemical screening

Entada africana ethanol stem bark extract was subjected to phytochemical analysis using standard protocol (Trease, 2009 and Sofowora, 1982).

2.4 Elemental Analysis

The crude powder (2g) was ashed in an oven at 60^{o}C for three hours, about 0.5g of the sample was then acid digested by addition of 10ml Hydrochloric acid (HCl), Nitric acid (HNO_3) and perchloric acid ($HCLO_4$) respectively. The flask was then heated in an electro thermal heater with gentle swirling till digestion completed by evolution of white fumes. The digested mixture was evaporated down to 5ml using rotator evaporator; they were then made up to 10ml with 2M HNO_3, and to which were added 30ml of distilled water and kept in a 100ml beaker. The resulting solution was used for the elemental analysis using atomic absorption spectrophotometer (AOAC, 1998).

2.5 Acute toxicity study

The median lethal dose (LD_{50}) of the extract was determined intraperitoneally in mice using the method described by Lorke (1983). 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) group of one (1) mouse each were further administered with doses of 15, 25, 40 and 60 mg/kg body weight. The mice were also observed for 24 hours, for signs and symptoms of toxicity including mortality. The LD_{50} 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.

2.6 Anti-diarrhoea studies

2.6.1 Castor oil-induced diarrhoea in mice

Method of Sunil *et al* (2001) and Appidi *et al* (2008) was used. The mice were fasted 12 hours prior to the commencement of the experiment and were randomly divided into five groups of five mice each. Mice in the first group were administered with 10ml/kg (*i.p*) normal saline, the second, third and fourth group were administered with 5, 2.5 and 1.25mg/kg of ethanol leaf extract of *Entada africana* (*i.p*) and the fifth group was administered with loperamide at 5mg/kg (*i.p)*. After 30 minutes of administration of normal saline, extracts and loperamide; castor oil 0.2 ml/mouse was administered intragastrically. The animals were then place 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 droppings. Their absence was recorded as protection from diarrhoea, and the percentage protection was calculated.

2.6.2 Magnesium sulphate induced diarrhoea in mice

Method of Sunil *et al* (2001) and Appidi *et al* (2008) was used. The mice were fasted 12 hours prior to the commencement of the experiment and were randomly divided into five groups of five mice each. Mice in the first group were administered with 10ml/kg (*i.p*) normal saline, the second, third and fourth group were administered with 5, 2.5 and 1.25 mg/kg of ethanol leaf extract of *Entada africana* (*i.p*) and the fifth group was administered with loperamide at 5mg/kg (*i.p*). After 30 minutes of administration of normal saline, extracts and loperamide; 2g/Kg magnesium sulphate was administered intragastrically to each mouse according to their body weight. The animals were then place 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 droppings. Their absence was recorded as protection from diarrhoea, and the percentage protection was calculated.

2.6.3 Gastrointestinal motility (Charcoal meal transit)

Method of Rouf *et al* (2003) was used. The mice were fasted 12 hours prior to the commencement of the experiment and were randomly divided into five groups of five mice each. Mice in the first group received 10 ml/kg (*i.p*) normal saline, the second, third and fourth groups received 5, 2.5 and 1.25mg/kg of ethanol leaf extract of *Entada africana* (*i.p*) and the fifth group was administered with loperamide at 5mg/kg (*i.p*). Five minutes after administration of normal saline, extract and loperamide, 0.5ml 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%. The same procedure was also applied to the stem bark and root bark extracts.

2.6.4 Isolated tissue studies

Method of Amos *et al* (2000) was used. An overnight starved rabbits (weighing 2kg) were sacrificed, exsanguinated and the abdomen open. Segment of the rabbit jejunum of about 2–3cm long was removed and mounted in a 25 ml organ bath containing Tyrode's solution at 37 ^{0}C and aerated with air. Thirty minutes equilibration period was allowed and the physiological solution was changed every 15 minutes. At the end of the equilibration period, the effects of the acetylcholine, extract and atropine sulphate on the rabbit jejunum were evaluated. The contact time for each concentration was maintained at 3 minutes and the tissue is washed three times after is equilibration period. The responses were recorded using Ugo Basile Microdynamometer 7050 with paper speed of 24 mm/min.

2.7 *Statistical analysis*

The data obtained were expressed as Mean ± SEM (standard error of mean) or percentages protection. Data were analyzed using one way analysis of variance (ANOVA), followed by Dunnett's post-hoc test. Values of $p \leq 0.05$ were considered statistically significant.

3.0 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 are absent (Table 1).

Table 1: Phytochemical Constituents of 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 Ethanol Stem Bark Extract of *Entada africana*

Elements	Concentration µg/L Stem Bark Extract	WHO Standard (µ/g)
Copper	2.3	100-300

Iron	18.8	50-5,000
Lead	42.7	5-30
Magnesium	1010.1	100-200
Manganese	12.9	100-20,000
Sodium	989.4	400-500
Zinc	6.4	150-20,000

3.3 Acute toxicity study

The median lethal dose of the *Entada africana* ethanol stem bark extract is 774.6mg/kg.

3.4 Castor-Oil Induced Diarrhoea

The stem bark extract produce a dose dependent increase in protection when compared with normal saline administered group. Stem bark 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 diarrhoeal droppings (Table 3).

Table 3: Effect of Ethanol Stem Bark Extracts of *Entada africana* on Castor Oil Induced Diarrhoea in mice

Treatment mg/kg	Dose (i.p)	Quantal protection	Protection (%)
Normal Saline	*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	100

3.5 Magnesium Sulphate – induced Diarrhoea

The stem bark extract produce a dose dependent increase in protection when compared with normal saline administered group. Stem bark 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 diarrhoeal droppings (Table 4).

Table 4: Effect of Ethanol Stem Bark Extract of *Entada africana* on Magnesium Sulphate-induced Diarrhoea in Mice

Treatment mg/kg	Dose (i.p)	Quantal protection	Protection (%)
Normal Saline	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	100

3.6 Gastrointestinal motility (Charcoal meal transit)

The stem bark extract at doses of 50, 100 and 200 mg/kg produce a significant ($p \leq 0.01$) inhibition of the charcoal plug in mice by (7.60±1.18, 6.70±2.28 and 6.12±1.17), when compared with normal saline administered group(33.50±1.79). Loperamide at a dose of 5 mg/kg produce significant ($p \leq 0.01$) inhibition of the charcoal plug in mice when compared with normal saline administered group (33.50±1.79) (Table 5).

Table 5: Effect of 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	*10.00	33.50 ± 1.79[a,b,c,d]	86.7
Extract	50.00	7.60 ± 1.18[a]	18.6
	100.00	6.70 ± 2.28[b]	15.7
	200.00	6.12 ± 1.17[c]	15.2
Loperamide	5.00	4.74 ± 2.47[d]	11.5

Data were analyzed using one way ANOVA followed by Dunnett post-hoc a = p≤ 0.05 and b = p≤0.01 n=5.

3.6 Effect Ethanol Stem Bark Extract of Entada africana and Propranolol on Isolated Rabbit Jejunum

The stem bark extract of *Entada africana* induced a concentrations (0.4-3.2 mg/ml) dependent relaxation of rabbit jejunum (Table 6a). The relaxation induced by Isoprenaline at concentration of 0.008 µg/ml was blocked by propranolol at concentration of 0.04 µg/ml; also the relaxation induced by the stem bark extract at concentration of 1.6 mg/ml was blocked by propranolol at concentration of 0.04 µg/ml (Table 6b).

Table 6a: Effect of *Entada africana* Ethanol Stem Bark Extract 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 *Entada africana* 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	0.97 ± 0.12	0.03 ± 0.03

	(0.04)	(1.6)

Values are expressed as Mean ± SEM, n=3

4.0 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* (2010); Bako *et al* (2005); Gidado *et al* (2013) and Njayou *et al* (2013). Mbatchou *et al* (2011) also reported the presence of alkaloids, cardiac glycosides, proteins, steroids, tannins and terpenoids in the extract. However, in contrast to the study of Tibiri *et al* (2010) 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 (Elujoba, 1989).

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 (Pednekar and Raman, 2013). The elemental analysis showed the presence of zinc, which is an important element responsible for many enzymatic processes (Shamaki *et al.*, 2012; Pednekar and Raman, 2013). 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 (Patel *et al*., 2005; Aggarwal et *al*., 2007). Zinc is also important for normal growth and development of children both with and without diarrhoea (Black and Sazawal, 2001; Bhatnagar and Natchu, 2004; Fischer *et al*., 2009). The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) in 2004 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.

Intraperitonial median lethal dose (LD_{50}) determination of the extract in mice showed that the extract is moderately toxic (774.6 mg/kg) according to LD_{50} classification (Lorke, 1983).

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 acts on presynaptic μ-receptor located on cholinergic nerve terminal of gut, thereby inhibiting the gut motility as well as reducing electrolyte and water secretion (Pasricha, 2006). Also the extract shows protection against magnesium sulphate induced diarrhoea by counteracting the increased osmotic imbalance caused by magnesium sulphate due to increase in electrolyte secretion (Lakshminarayana *et al.*, 2011.)

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.

The isolated tissue study showed that extract exhibited a concentration-dependent relaxant activity on rabbit jejunum similar to that produced by standard drug isoprenaline, a non-selective beta receptor agonist. There are two possibilities for the mechanism by which the relaxant effect occur, either through adrenergic pathway or direct smooth muscle relaxant effects. 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. Flavonoids have been known to inhibit contractions induced by spasmogenes (Macauder, 1986). The presence of flavonoids in the plant extract could be responsible for the observed concentration-dependent relaxation of the rabbit jejunum. The observed relaxation property 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 (Longanga-Otshudi *et al*., 2000). Flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) (Christopher *et al*., 1996; Haruna *et al*., 1997). Certain flavonoids inhibit inflammatory processes by inhibiting key enzymes involved in the synthesis of prostaglandins (Manthey *et al*., 2001), Flavonoids were reported to possess antidiarrhoeal activity which is attributed to their ability to inhibit intestinal motility and hydro-electrolytic secretion (Ndukui *et al*., 2013). Flavonoids and terpenoids derivatives are known for inhibiting release of autacoids and prostaglandins, thereby inhibiting the motility and secretion induced by castor oil (Nigam and Paarakh, 2013).

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+} (Belemtougri *et al*., 2006). 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.

5.0 Conclusion

Ethanol stem bark extract of *Entada africana* possess antidiarrhoeal activity against castor oil and magnesium sulphate induced diarrhoea in mice. However, the extract showed more activity in magnesium sulphate induced diarrhoea. This justifies the use of this plant in the management of diarrhoea in traditional medicine.

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