Original Research Article

Antidiarrhoeal effects of hydromethanolic leaves extract of *Ipomea asarifolia* in albino rat model

ABSTRACT

Aim: To evaluate the antidiarrhoea effect of hydromethanolic leave extract of *I. asarifolia* (HLEIA) on castor oil-induced diarrhoea

Place and Duration of Study: Department of Biochemistry, Faculty of Life sciences, Kebbi State University of Science and Technology, Aliero, Kebbi state, Nigeria. P.M.B.1144. Kebbi State. Nigeria, between February 2015 and September 2016.

Methodology: In a continuous effort to search for bioactive agents from medicinal plants, the antidiarrhoea activity of *I. asarifolia* was investigated. The effect of hydromethanolic leave extract of *I. asarifolia* (HLEIA) on castor oil-induced diarrhoea, gastrointestinal transit and intestinal fluid accumulation (enteropooling) were respectively assessed ~~respectively~~ in albino rats. Qualitative phytochemical analysis ~~were~~was carried out using standard procedures while acute oral toxicity ~~toxicity~~ studies was determined using the staircase method.

Results: The phytochemical analysis showed the presence of alkaloid, terpenoid, tannin, saponin, phenols. The LD_{50} was estimated to be greater than 3000 mg/kg since there ~~is~~ was no mortality recorded after 14 days of acute oral toxicity studies. Sub-chronic administration of graded doses (150 – 600 mg/kg) of HLEIA significantly ~~showed significant~~ ($p < 0.05$) reduced diarrhoea episodes, decreased ~~in~~ gastro intestinal movement and inhibited intestinal fluid accumulation ~~in treated animals respectively~~ compared ~~with~~ to the control. The antidiarrhoea effect of treated group (600mg/kg–) was comparable to that of the standard drug L~~l~~operamide.

Conclusion: The findings of the present study scientifically validate the use of *I. asarifolia* in the treatment of diarrhoea.

Keywords: *Gastro-intestinal transit, percentage inhibition, Castor oil, enteropooling, loperamide, diarrhoea episodes*.

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1. INTRODUCTION

The use of plants for medicinal purposes is an age old tradition in Africa, Asia and Latin America [1, 2]. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain [3]. The striking coincidence between indigenous medicinal plants uses and scientifically-proved phytochemical and pharmacological properties shows that the traditional remedies are an important and effective part of indigenous healthcare systems which is totally dependent on traditional healers [4]. Growing interest on the use of medicinal plants for primary health care is greatly influenced by the rising cost and side effects associated with most modern drugs. Modern pharmacopoeia still contains at least

25% of drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants.

Ipomoea asarifolia (Convolvulaceae) is a glabrous succulent perennial plant trailing on the ground. It is found throughout West Africa and is a common weed of hydromorphic soils, low lying and inland valleys, streams and river banks. In Nigeria, the traditional names include "Duman kada" in Hausa and "Gboro ayaba" in Yoruba [5]. Various parts of the plant are used by traditional medicine practitioners in Nigeria for the management and treatment of several disorders which include opthalmia, neuralgia, headache, arthritic pains and stomach ache. In Kebbi (North- West Nigeria), *Ipomea asarifolia* has been widely used for the treatment of various stomach disorders of which diarrhoea is the most common.

Diarrhoea is a leading cause of malnutrition and globally, there are nearly 1.7 billion cases of childhood diarrhoeal disease every year [6]. –It is a very common ailment and national problem in many tropical countries and the cause of 4-5 million deaths throughout the world annually [7]. Diarrhoea remains the second leading cause of death among children under five globally [8]. Nigeria was estimated to have a total number of annual child deaths due to diarrhoea to be 151,700 [8]. Diarrhoea may be caused by a wide array of agents such as entero-pathogenic microorganisms (*Shigella flexneri* and *Shigella dysenteriae*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*), alcohol, irritable bowel syndrome, bile salts, hormones, secretory tumors and intoxication [9,10]. Dependency on plants as medicine in controlling diseases is common among rural populace in Nigeria because of its relative safety and affordability compared with the cost of conventional medicines. Therefore, there is need to provide scientific bases of justification on the therapeutic uses of medicinal plants against infectious diseases. *Ipomea asarifolia* has been used in traditional medicine for treating various ailments, including diarrheal, without scientific verification of its effects. The present study was therefore designed to validate this claim of *Ipomea asarifolia* in the treatment of diarrhoea by the communities in Kebbi State, Northwest Nigeria.

2. MATERIAL AND METHODS

2.1 Plant collection

The fresh leaves of *Ipomea asarifolia* were collected in the month of March, 2015 at Kebbi State University of Science and Technology, Aliero (KSUSTA) main campus. The plant was identified taxonomically and authenticated at the Department of Biological Science, Kebbi State University of Science and Technology Aliero, Nigeria with a voucher specimen no 001.

2.2 Plant extraction

The collected leaves of *I. asarifolia* were air-dried and then grounded into powder. 200g of the powdered leave was macerated in methanol for 72 hours, filtered using muslin cloth and dried in an oven at 45 ^{o}C. The percentage yield of the hydromethanolic extract of *I. asarifolia* was 32.62%.

2.3 Animals

Albino rats were used for the study. They were purchased at the animal house of Usmanu Danfodio University Sokoto, Sokoto State. All the animals were kept in the cage and allow acclimatizing for one week in Biochemistry Laboratory of Kebbi State University of Science and Technology Aliero, Kebbi State, before the experiment started. The animals were fed with standard pellet diet and water. The container for the food and water were washed and cleaned daily as food and water were renewed every day to ensure hygiene and maximum comfort for the animal.

2.4 Phytochemical screening

The presence of various phytochemical constituents in the extract was determined using the standard screening tests [11].

2.5 Lethal dose determination (LD_{50})

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Authors have to indicate the exact solvent used, its volume and the ratio in the case the mixture of water and methanol was used

Comment [y5]: See Results Sction. What is the exact extraction yield? 32.62 or 32.95%? If one or other, There is a doubt, from 200 g, how could authors succeded to obtain approximately up to 33%?

The up and down procedure as described by Dixon [12] was used to evaluate the oral acute toxicity of hydromethanolic leaves extract of *I. asarifolia.* Five non-pregnant adult albino rats ~~were~~ randomly selected from the pull of acclimatized rats were used for this experiment. The animals were weighed ~~individually~~, marked and individually housed ~~individually~~ in cages prior to treatment. The rats to be treated were fasted overnight but allowed free access to water. Freshly prepared hydromethanolic leaves extract of *I. asarifolia* was administered orally at a limited dose of 3000 mg/kg. The first animal was dosed and observed for sign of toxicity or death. If the animal ~~survives~~survived, the same procedure was adopted until all the five rats were dosed and observed for 48 hours for signs of acute toxicity, morbidity and mortality for the first 48 hours and up to 14 days. The behavioral changes and other changes observed in animals were recorded according to Organization for Economic and Cultural Development (OECD) 425 guidelines [12].

2.6 Antidiarrhoea studies

2.6.1 Gastrointestinal motility test

Rats were fasted for 18 h and divided into five groups of five animals each~~.~~ Group I received 5 m~~l~~L/kg normal saline orally, group II received Loperimide (5 mg/kg~~,~~), group III - V received hydromethanolic leave extract of *Ipomea asarifolia* (150, 300 and 600 mg/kg~~,~~) respectively~~.~~. After 1 ~~hour~~ h. of administration, 1 ~~ml~~ mL of ~~de~~activated charcoal meal was administered to all the rats. Thirty ~~30~~ minutes later, each rat was sacrificed and the small intestine ~~was~~ removed. The total length of the intestine and the distance moved by the charcoal meal from the pylorus to the caecum was measured (cm). The intestinal charcoal transit was expressed as a percentage of the distance moved by charcoal to the length between pylorus and the caecum [13] and was calculated according to the following formula.

$$\% \text{ Inhibition} = \frac{\text{distance travelled by charcoal meal in control group} - \text{treated group}}{\text{Distance travelled by charcoal meal in group}} \times 100$$

2.6.2 Castor oil induced diarrhoea

Twenty rats were fasted for 18 ~~hours~~ h and divided into five groups of five animals each. Castor oil ~~at a dose of~~(1 ~~ml~~ mL) was orally given ~~orally~~ to all groups of animals for the induction of diarrhoea. Thirty minutes after castor oil administration various treatments were given~~,~~. Group I (control) animals were treated with normal saline (5 ~~ml~~mL/kg), Group II animals were treated with ~~loperamide~~ Loperamide (5 mg/kg), a positive control. Group III-V ~~served~~ was treated ~~and~~ with hydromethanolic extract of *Ipomea asarifolia* (150, 300 and 600 mg/kg) ~~were~~ orally administered ~~respectively orally~~. Animals were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the severity of diarrhoea was assessed hourly for 6 hours [14]. The total score of diarrhoea faeces for the control group was considered as 100%. The results were expressed as a percentage of inhibition of diarrhoea.

2.6.3 Castor oil induced enteropooling

Intraluminal fluid accumulation was determined by the method ~~of~~ described by Robert *et al* [15]. Rats were divided into five groups of four animals each~~,~~. ~~one~~ One hour before oral administration of castor oil (2 ~~ml~~mL/rat~~.~~), group I orally received ~~Normal~~ normal saline, ~~orally~~ (5 mg/kg), and served as control~~.~~ ~~Group~~ group II animals received Loperamide (5 mg/kg) while groups III – V through oral intubation, respectively received the plant extract ~~of~~ at ~~doses of~~ 150, 300 and 600 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.

2.7 Statistical analysis

Data was expressed in as mean standard error of mean (SEM) and statistical analysis ~~was~~ were carried out employing one way analysis of variance (ANOVA) followed by Dunnett multiple comparisons test at $p<0.05$ significance level using Graphpad software, San Diego California USA, (www.graphpad.com).

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3. RESULTS AND DISCUSSION

The percentage yield of Hydromethanolic leaves extract of *Ipomea asarifolia* (HLEIA) was found to be 32.95%. The high percentage yield of HLEIA suggests that the plant is a good source of extract since it contains sufficient amount which could be subjected further for isolation studies.

In the acute oral toxicity studies, it was observed that oral administration of HLEIA to the rats at 3000 mg/kg neither caused no mortality nor any apparent signs of toxicity in the animals within the first 24 hours and up to 14 days after its administration. This indicates that, the lethal median dose (LD_{50}) of the extract~~s~~ is greater than 3000 mg/kg suggesting the plant extract ~~is~~ may be safe for consumption as herbal formulation (Reference).

One hour after castor oil administration, all the rats in the control group ~~of animals~~ produced copious diarrhoea. HLEIA produced a marked anti-diarrhoea effect in the rats, as shown in Table 1. At ~~dose of~~ 150 mg/kg, the extract significantly (p<0.01) decreased ~~(p<0.01)~~ the total number of wet faeces produced upon administration of castor oil compared with control group. Highest inhibition percentage ~~inhibition~~ of defecation ~~in the extract treated groups~~ was observed with the extract at 150 mg/kg ~~of the extract~~ (40.00%) ~~while~~ and with ~~the~~ Loperamide ~~treated group retained the maximum percentage inhibition of defecation~~(64.62).

Table 1: Effect of HLEIA on castor oil induced diarrhoea in albino rats~~.~~

Treatment	Total number of faeces	Number of diarrhoea faeces	% Inhibition of diarrhoea
Normal saline(5 mg/kg)+ castor oil (2 ~~ml~~mL)	22.25 ± 2.66	16.25 ± 2.18	-
Loperamide (5 mg/kg)+ castor oil (2 mL~~ml~~)	13.00 ± 0.91	5.75 ± 0.63**	64.62
HEIA (150 mg/kg)+ castor oil (2 mL~~ml~~)	20.50 ± 0.87	9.75 ± 0.63**	40.00
HEIA (300 mg/kg)+ castor oil (2 mL~~ml~~)	24.00 ± 0.91	11.25 ± 0.63*	30.77
HEIA (600 mg/kg)+ castor oil (2 mL~~ml~~)	22.50 ± 0.87	11.00 ± 0.41*	32.31

Values are expressed as mean ± S.E.M; (n=5) in each group. Data were~~as~~ analyzed by one way ANOVA followed by Turkey-Kramer multiple comparisons test. *P<0.05 and **P<0.01 when compared to the control. HEIA=Hydromethanolic extract of Ipomoea asarifolia.

Several studies have shown that prior administration with some plant extract had protective effect on the intestinal tract. These studies have validated the use of antidiarrhoea medicinal plants by investigating the biological activity of extracts of such plants which have antispasmodic effects, delayed intestinal transit, ~~suppressed~~ gut motility, stimulate water adsorption, or reduce the intraluminal fluid accumulation [16,17,18,19].

In antimotility test, ~~S~~sub-chronic administration of graded doses of HLEIA showed significant ~~difference~~ effects ~~(p<0.05)~~ in treated animals receiving plant extract at 300 mg/kg (p<0.01) or at ~~500mg~~600 mg/kg (p<0.05) respectively compared with the control (Table 2). There was also a significant increase (P<0.01) in percentage intestinal transit in the drug-treated group when compared with the control. The anti-diarrhoea effect of treated group receiving 300 mg/kg was comparable with that of the standard drug ~~loperamide~~Loperamide.

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Table 2: Gastro intestinal motility effect of HLEIA in albino rats.

Treatment	Length of intestine (cm)	Distance moved by charcoal meal (cm)	% Inhibition
Normal saline(5 mg/kg) + castor oil (2 mL)	86.03± 2.78	45.45 ± 2.56	0.00
Loperamide (5 mg/kg) + castor oil (2 mL)	90.00 ± 4.44	9.50 ± 3.43**	79.10
HEIA (150 mg/kg) + castor oil (2 mL)	82.25 ± 2.75	37.75 ± 8.41	16.94
HEIA (300 mg/kg) + castor oil (2 mL)	87.35 ± 3.65	14.63 ± 1.55**	68.10
HEIA (600 mg/kg) + castor oil (2 mL)	84.88 ± 3.33	21.80 ± 5.29*	52.04

Values are expressed as mean ± SEM from the experiment. Data analysedanalyzed by one way ANOVA, using Dunnets comparism test. *(P< 0.05) and ** (P < 0.01) significantly difference when compared with control group.

Gastrointestinal motility test with activated charcoal was carried out to find the effect of the hydromethanolic extract of *I. asarifolia* on peristalsis movement. The result shows that HLEIA (300 mg/kg) was found to be comparable with the standard drug Loperamide, a drug which is widely used for the treatment of diarrhoea. Loperamide is known to exert its antidiarrhoea activity by regulating the gastrointestinal tract, slowing down motility in the intestine, reducing colon flow rates and consequently any effect on colonic motility [20].

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Castor oil caused accumulation of water and electrolytes in intestinal loop. HLEIA compare with the control, significantly ($P < 0.01$)) and dose dependently inhibited castor oil-induced enteropooling in rats (Table 3). The inhibition rates for the extract were at oral dose of24.02, 41.69 and 600 mg/kg (50.53 %) respectively at 150, 300 and 600 mg/kg in a dose dependent manner compare with the control (Table 3). The intestinal fluid in control animals was 2.83 ± 0.48 mlmL. The iInhibition of intestinal accumulation was were 24.02%, 41.69% and 50.53% respectively at doses 150, 300 and 600 mg/kg respectively. The standard drug loperamide Loperamide (5 mg/kg), also significantly inhibited intestinal fluid accumulation (60.07 %).

Table 3: Enteropooling effect of HLEIA in albino rats.

Treatment	Volume of intestinal fluid (mL)	% Inhibition
Normal saline(5 mg/kg) + castor oil (2 mlmL)	2.83 ± 0.48	--
Loperamide (5 mg/kg) + castor oil (2 mL)	1.13 ± 0.10**	60.07
HEIA (150 mg/kg) + castor oil (2 mL)	2.15 ± 0.16	24.02
HEIA (300 mg/kg) + castor oil (2 mL)	1.65 ± 0.06*	41.69
HEIA (600 mg/kg) + castor oil (2 mL)	1.40 ± 0.04**	50.53

Values are expressed as mean ± S.E.M; (n=5) in each group. Data was were analyzed by one way ANOVA followed by Turkey-Kramer multiple comparisons test. *P<0.05 and **P<0.01 when compared to the control. HEIA=Hydromethanolic extract of *Ipomoea asarifolia*.

Castor oil produces diarrhoea due to its most active metabolite, ricinoleic acid by hypersecretory response, which stimulates peristaltic activity in the small intestine, leading to

changes in the electrolyte permeability of the intestinal mucosa [21]. Ricinoleic acid causes irritation and inflammation of the intestinal mucosa leading to the release of prostaglandins which stimulate hyper-motility, alteration in the electrolyte permeability of the intestinal mucosa and increase in the volume of intestinal contents by preventing the reabsorption of sodium, potassium and water [22, 23, 24]. In the present study, HLEIA showed a dose-related anti-enteropooling effect via reduced volume of the intestinal contents and also significantly inhibited castor oil-induced diarrhoea in rats by the significant reduction of the number of diarrhoeal episodes and total faeces. This implies that the extract probably enhanced the absorption of electrolytes and water from the intestinal lumen, while reducing the rate of their secretion into the small intestine or has the ability to inhibit the castor oil-induced intestinal accumulation of fluid in a manner similar to the standard anti-diarrhoeal drug (~~loperamide~~Loperamide).

In the phytochemical analysis, HLEIA showed the presence of alkaloids, saponins, terpenoids, tannins, phenols, steroids and resins. The need for phytochemical screening has become imperative since many plants accumulate biologically active complex organic chemicals (secondary metabolites) in their tissues. ~~*Ipomoea asarifolia* revealed the presence of alkaloid, terpenoids, resins, saponin, steroids, and phenols~~. Previous reports have demonstrated that~~e~~ antidiarrhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, terpeniods, flavonoids and sterols [25, 26, 27, 28, 29]. It could therefore be suggested that the secondary metabolites present in *I. asarifolia* could be responsible for the pharmacological effects observed.

4. CONCLUSION

The present study reveals that hydromethanolic leaves extract of *I. asarifolia* contains phytocontistuents such as alkaloids, terpenoids, resins, tannin, saponin, phenols and steroids that are ~~rich in~~known for their antidiarrhoeal properties. The result obtained in this research establishes its efficacy and scientifically validate the use of *I. asarifolia* in the treatment of diarrhoea. Further research need to be undertaken to isolate and purify the bioactive components of this plant.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICAL APPROVAL

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

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