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Plausible Mechanisms of Anti-diarrhoeal Action of Hydromethanolic Extract of *Leptadenia hastata* (Pers.) Decne Leaves

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Abstract

Objective: The mechanism(s) of anti-diarrhoeal action of the Hydromethanolic Extract of *Leptadenia hastata* Leaves (HELH) was investigated in this study.
Materials and Methods: Preliminary phytochemical analysis and acute toxicity evaluation of the extract were carried out according to standard methods. Experimental diarrhea was induced in rats with castor oil, and the effect of the extract on castor oil-induced gastrointestinal motility and enteropooling was consequently investigated. Anti-microbial screening of the extract was also carried out.
Results: Phytochemical analysis of detected the presence of alkaloids, phenols, flavonoids, terpenes, tannins and saponins in the extract**.** In the acute toxicity study, the extract produced no signs of toxicity or mortality in rats up to a dose of 5000 mg/kg. The oral LD_{50} of the HELH was taken to be >5000mg /kg. In the antidiarrheal study, the extract significantly ($p<0.05$) and dose-dependently decreased the frequency of defecation, the number of unformed feces, distance travelled by activated charcoal in the gastrointestinal tract and slightly reduced the weight of intestinal contents of treated rats compared to control. Antibacterial screening of HELH showed a concentration- dependent growth inhibition for both *E. coli* and *Staphylococcus aureus*.
Conclusion: It was therefore concluded that the mechanisms by which the leaf extract of *L. hastata* exerts its antidiarrheal effect are by reduction of gastrointestinal motility, anti-secretory and anti-microbial activities

Keywords: Mechanism, Hydromethanolic, anti-diarrhoeal, *Leptadenia hastate*

Introduction

Diarrhoea is defined as the abnormal passage of loose or liquid stools more than three times daily and/or a volume of stool greater than 200 g/day. Diarrhoea is a common symptom of gastrointestinal infections. Acute diarrhea being the most common is usually caused by an infectious agent, even though drugs, poisons or acute inflammatory reactions are contributing factors (Thapar & Sanderson, 2004). Rotavirus is the major causative agent of infectious diarrhea, particularly in young children now a days, however, other viral (*Enterovirus*, *norovirus* and adenovirus), bacterial (*Salmonella* sp., *Shigella* sp., *Escherichia coli*, *Camphylobacter* and *Vibrio cholerae*) and parasitic (*Cryptosporidium* and *Giardia*) agents are important pathogens (Allen *et al.*, 2004). Diarrhoea is more prevalent in the developing world largely due to the lack of safe drinking water, sanitation and hygiene, as well as poorer overall health and nutritional status. In Nigeria, diarrheal infection remains the number one killer disease among children under 5 years, while 7-12 month old babies remain the most susceptible (Audu *et al.*, 2004).

Although a diarrhea disease control programme (DDC) has been launched by the World Health Organization (W.H.O), diarrhoea is still a big public health challenge in developing countries. Consequently, the use of herbal drugs in the treatment of diarrhea is a common practice in many developing countries. In Nigeria, dependency on plants as treatment for diarrhoea is common among rural populace 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 these plants. The present study was therefore designed to validate this claim of *Leptadenia hastata* in the treatment of diarrhoea by the communities in Kogi State, North Central Nigeria and possibly investigate its mechanism(s) of action.

Leptadenia hastata (Pers.) Decne belongs to the family Asclepiadaceae widely used in Tropical Africa as vegetable (Burkil, 1985). The plant is medicinally important in the treatment of many ailments (Burkil, 1985; Oliver-Boyer, 1986; Aliero *et al.*, 2001). Ethnobotanical information obtained from traditional medical practitioners in northern. Akuba *et al.*, (2019) reported the hepatoprotective effect of the plant against alcohol- induced liver injury in rats. The antibacterial and antimicrobial effects of *L. hastata* have been reported (Aliero and Wara, 2009) and the result of its toxicity studies showed that the plant is relatively safe (Tambuora *et al*., 2005).

Materials and Methods

Materials

Chemicals and drugs

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) while the drugs were purchased from a local pharmacy shop.

Animals

Adult Wistar rats of either sex weighing 180–220g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. 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 animals.

Methods

Plant Collection and Identification

The fresh leaves of *Leptadenia hastata* were collected in the month of March, 2018 at Okpella area of Edo State, Nigeria. The plant was identified taxonomically and authenticated at the herbarium unit of Biological Sciences Department, Federal University, Lokoja where a voucher specimen was deposited for future reference.

Figure 1: A picture of *Leptadenia hastata* in its natural habitat

Extraction Procedure

The collected leaves of *L. hastata* were air-dried and then ground into powder. 1000g of the powdered leaves was macerated in 5000 ml of methanol: Water (70:30) for 72 hours, filtered using muslin cloth and dried in an oven at 45ºC. The extract was labelled 'HELH' (Hydromethanolic Extract of *Leptadenia hastata*) and stored in the refrigerator till required for analysis.

Preliminary Phytochemical Screening

The presence and absence of secondary plant metabolites in the crude Hydromethanolic leaf extract of *L. hastata* was screened by color forming and precipitation assays using standard procedures (Trease & Evans, 1989; Tiwari *et al.*, 2011).

Acute Toxicity Study/ LD_{50} Determination

The LD_{50} of the extract was estimated by procedure described according to the method of Lorke (1983) with modification. Twenty one (21) Wistar rats were divided into seven (7) groups of three (3) animals per group. Doses of 10, 100, 1000, 2000, 3000, 4000 and 5000 mg /kg were administered orally to the groups respectively. The treated animals were monitored for 24 h, for mortality and general behavioral characteristic indicative of animal toxicity. The LD_{50} was then estimated by taking the square root of the least dose that killed all the animals, and the highest dose that do not kill any animal/s or the geometric mean of the lowest dose causing death and the highest dose causing no death. That is, LD_{50} is equal to (highest dose causing no death multiply by lowest dose causing death) $^{1/2}$

Experimental Design

Castor oil-induced diarrhea

The method of Offiah and Chikwendu, (1999) was adopted. Twenty- five (25) rats of both sexes were fasted overnight but allowed free access to water. They were randomized into five groups of five rats each. Group I served as control and were administered 2 ml normal saline (0.9%). Groups II- IV were administered 100, 200 and 400 mg/kg of HELH orally while Group V was administered diphenoxylate hydrochloride (5 mg/kg) intraperitoneally. All rats were housed singly in a cage lined with white blotting paper. 1 h after treatments, each of the rats was treated with 1 ml of castor oil orally. Rats were then observed for 6 h and the number of water (wet) feces counted via fecal spots on the white bloated paper lining the cage where individual rat was kept. Percentage protection was calculated as follows:

$$\%\,\text{Protection} = \frac{\text{Mean number of defecation} - \text{Mean number of treated group}}{\text{Mean number of defecation of control}} X\,100$$

Effect of castor oil-induced gastrointestinal motility

The method of Chitme *et al.* (2004) was adopted. Rats were fasted overnight and then randomized into five groups of five rats each and allowed free access to water. Group I served as control and was administered 2 ml normal saline (0.9%) orally while group V was administered 3 mg/kg of atropine intraperitoneally. Groups II-IV was administered 100, 220 mg/kg, and 400 mg/kg of the HELH orally. After 10 min of administering the extract and drug, 1 ml of 5% activated charcoal suspension in 10% aqueous solution of Acacia powder was administered to treated rats. Rats were then sacrificed 30 min later and the abdomen was opened to measure the distance travelled by the activated charcoal. The results were expressed as percentage of the total length of the intestine from the pylorus to the caecum.

Effect of castor oil-induced enteropooling

The method of Robert *et al.* (1976) was adopted. The intraluminal fluid accumulation due to the effect of castor oil was determined. Rats were fasted overnight t but allowed access to fresh drinking water. The rats were randomized into five groups of five rats each. Group I served as control and was administered 2 ml normal saline. Groups II- IV were administered 100, 200 and 400 mg/kg of HELH orally. Group V was administered atropine (3 mg/kg) intraperitoneally. An hour later, 1 ml of castor oil was administered to each of the treated rats. They were then sacrificed after 1h post castor oil administration. The small intestines were removed, tied at both ends with thread and weighed. Intestinal contents were collected by milking and the volume measured.

Antimicrobial tests

The antimicrobial activity/screening of HELH was tested using the streak plate bore-hole method. The organisms were grown in nutrient agar and plant extract with concentration ranging from 1.0% to 10.0% (w/v) with sterile distilled water were used for

the antimicrobial analyses. A sterile inoculating loop was used to streak the organisms over the surface of the medium. Different concentrations of plant extract were impregnated into wells using sterile pipettes. The plates were incubated for 24 h and the zones of inhibition around the wells were measured.

Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Fishers test and difference between means at $P< 0.05$ were considered significant.

Results

Phytochemical Analysis

Results detected the presence of Alkaloids, phenols, flavonoids, steroids, terpenes, tannins and saponins in the extract **(Table 1)**.

Table 1: Qualitative Phytochemical Composition of the Hydromethanolic Extract of *Leptadenia hastata* Leaves (HELH)

Phytochemicals	HELH
Alkaloids	+++
Phenols	++
Flavonoids	+++
Steroids	++
Glycosides	ND
Terpenes	++
Tannins	+++
Saponins	++
Anthraquinones	ND

Key: + Slightly present, ++ moderately present, +++ highly present, ND- Not detected

Acute Toxicity Study

The results of acute toxicity studies showed no mortality or signs of toxicity up to a dose of 5000 mg/kg of HELH
(Table 2). The oral LD_{50} of the extract was therefore taken to be > 5000 mg/kg.

Table 2: Observed Effects of the Hydromethanolic Extract of *Leptadenia hastata* Leaves (HELH) on Rats

Treatment (mg/kg)	D/T	Observed Sign of Toxicity
10 mg/kg HELH	0/3	-
100 mg/kg HELH	0/3	-
1000 mg/kg HELH	0/3	-
2000 mg/kg HELH	0/3	-
3000 mg/kg HELH	0/3	-
4000 mg/kg HELH	0/3	-
5000 mg/kg HELH	0/3	-

Key: D= Number of deaths, T= Number of treated animals

Castor Oil- induced Diarrhea in Albino Rats

The frequency of defecation by the rats within 6 h of administration of HELH and castor oil is presented in **Table 3**. The extract produced a significant ($p<0.05$) and dose- dependent decrease in the frequency of defecation compared to control. The 400 mg/kg HELH- treated rats had the lowest frequency of defecation and the highest percentage of inhibition (63.86%) followed by the 200 and 100 mg/kg HELH-treated groups with 52.84 and 38.74% of inhibition respectively. The percentage inhibition (63.86%) of the highest dose (400 mg/kg) of the extract used was comparable to that of the standard drug- diphenoxylate hydrochloride (66.00%).

Table 3: Effect of Hydromethanolic Extract of *Leptadenia hastata* Leaves (HELH) on Castor Oil- induced Diarrhea in Albino Rats

Group	Treatment (mg/kg)	Mean number of Defecation in 6 h	Percent Protection (%)
I	Control	8.44±1.48	-
II	HELH 100mg/ kg+ CO	5.17±1.22[a]	38.74
III	HELH 200mg/ kg+ CO	3.98±0.88[a]	52.84
IV	HELH 400mg/ kg+ CO	3.05±0.75[a]	63.86
V	Diphynoxylate + CO	2.87±0.31[a]	66.00

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Fisher's test, (n=5). [a] Statistically significant at $p< 0.05$. CO= Castor oil

Gastrointestinal Motility

The effect of the extract on gastrointestinal transit of activated charcoal is shown in **Table 4**. A rapid movement of charcoal was observed in the control group. However, the rate of movement of charcoal was significantly ($p<0.05$) reduced in rats treated with HELH in a dose- dependent manner. The 400 mg/kg HELH –treated rats had a charcoal movement rate comparable to the standard (3 mg/kg atropine) group.

Table 4: Effect of Hydromethanolic Extract of *Leptadenia hastata* Leaves (HELH) on Charcoal Gastrointestinal Transit in Albino Rats

Group	Treatment (mg/kg)	Length of Intestine (cm)	Distance Travelled by Charcoal (cm)	Percent Intestinal Transit (%)
I	Control	38.3±1.23	32.4±3.23	84.60
II	HELH 100 mg/ kg+ Ch	35.3±1.38	22.4±3.47	63.46[a]
III	HELH 200 mg/ kg+ Ch	36.4±1.17	20.8±3.40	57.14[a]
IV	HELH 400 mg/ kg+ Ch	34.7±1.02	15.4±1.87	44.38[a]
V	Atropine 3mg/kg + Ch	38.4±1.34	16.3±1.96	42.43[a]

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Fisher's test, (n=5). [a] Statistically significant at $p< 0.05$. Ch = charcoal

Castor oil-Induced Enteropooling

Table 5 shows the effect of the extract on castor oil-induced enteropooling. A significant decrease ($p<0.05$) in the volume of intestinal contents was observed between the treated groups and control. The extract at a high dose (400 mg/kg) had a very pronounced effect which was higher even compared to that of the standard- atropine (3 mg/kg). The 100 mg/kg treated group had the highest percentage intestinal fluid inhibition of 61.97% followed by the group treated with 200 mg/kg HELH while the group treated with 400 mg/kg had the least percentage intestinal fluid inhibition (17.96%).

Table 5: Effect of Hydromethanolic Extract of *Leptadenia hastata* Leaves (HELH) on Castor oil-induced Diarrhea in Albino Rats

Group	Treatment (mg/kg)	Wt. of Full Intestine (g)	Wt. of Empty Intestine (g)	Wt. of Intestinal Content (g)	Percentage Inhibition of Fluid (%)
I	Control	5.28±0.66	2.44±0.36	2.84±0.23	-
II	HELH 100 mg/ kg+ Ch	4.99±0.78	3.91±0.23	1.08±0.11[a]	61.97
III	HELH 200mg/ kg+ Ch	5.01±0.86	3.70±0.28	1.31±0.29[a]	53.87
IV	HELH 400mg/ kg+ Ch	4.86±0.63	2.53±0.31	2.33±0.27[a]	17.96
V	Atropine 3mg/kg + Ch	5.15±0.52	2.90±0.27	2.25±0.30[a]	20.77

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Fisher's test, (n=5). [a] Statistically significant at $p< 0.05$. Ch = Charcoal

Antimicrobial Screening

Antibacterial screening of HELH showed a concentration dependent growth inhibition for both *E. coli* and *Staphylococcus aureus* (**Table 6**). The zones of inhibition remained clear without bacterial re-growth after 72 hours.

Table 6: Growth Inhibition of *E. coli* and *Staphylococcus aureus* by Hydromethanolic Extract of Leptadenia hastata Leaves (HELH)

Conc. Of Extract (%)	Zone of Inhibition	
	E. coli	***Staph. aureus***
1.00	8.29 ± 0.21[a]	7.01± 0.35
2.50	9.51 ± 0.33[a]	10.32 ± 0.71
5.00	12.14 ± 0.26[a]	14.42 ± 0.62
10.00	15.11 ± 0.39[a]	16.60 ± 0.81

Data are presented as mean ± SD

Discussion

The present study sought to assess the mechanisms of antidiarrheal activity of the Hydromethanolic leaf extract of *Leptadenia hastata*. In the acute toxicity study, no mortality and signs of toxicity such as changes in general behaviours and variations in body weight was observed with the extract up to a dose of 5000 mg/kg (Table 2). Based on the result, it can be stated that the oral LD_{50} value of the Hydromethanolic leaf extract of *L. hastata* is greater than 5000 mg/kg and this plant can be classified as 'safe' according to Lorke's method. This implies that the extract can be administered with some degree of safety, especially through oral route.

In the phytochemical analysis, the presence of alkaloids, saponins, terpenoids, tannins, phenols, flavonoids and steroids was detected (Table 1). The need for phytochemical screening has become imperative since many plants accumulate biologically active complex organic chemicals (secondary metabolites) in their tissues. Previous reports have demonstrated that antidiarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, terpenes, flavonoids and sterols (Gevid *et al.*, 2005; Balaji *et al.*, 2012; Das *et al.*, 2014). It could therefore be suggested that the secondary metabolites present in *L. hastata* could be responsible for the pharmacological effects observed as discussed below.

Castor oil induces diarrhoea via the action of its most active metabolite- ricinoleic acid which causes the irritation and inflammation of the intestinal mucosa leading to prostaglandins (PGE2α) release. The released PGE2 stimulates gastrointestinal motility and secretion of water and electrolytes (Longana *et al.*, 2000), thus inducing an increase in the peristalsis and an intestinal hyper secretion of fluid. The inhibition of prostaglandins biosynthesis prolongs the time of induction of diarrhea by castor oil (Rajat *et al.*, 2013).

In this study, the extract significantly and dose-dependently protected against diarrheoa by increasing the latency time and bringing about a decrease in the frequency of defecation (Table 3). There was also a significant reduction of total fresh weight of deposit, of water content and of the surface of impregnation of deposit. The anti- diarrhoeal effect of the extract is similar to that of diphenoxylate used as standard drug. The antidiarrheal activity of diphenoxylate results from its antispasmogenic and antisecretory properties on the intestine (Lenika *et al.,* 2005). It is safe to say therefore, that the extract through the phytochemicals it contains might have acted in a similar way- antispasmogenic and antisecretory properties on the intestine. Quercetin, a flavonoid relaxes smooth muscles and inhibits bowel contraction, probably by inhibiting intracellular calcium release from the sarcoplasmic reticulum (Morales *et al.,* 1994). Triterpenes have also been shown to have antispasmodic activities, as well as aid in the reabsorption of water in the intestine (Offiah *et al.,* 1996).

Results of this study also showed that *L. hastata* significantly ($p<0.05$) produced a reduction in the progression of charcoal meal and in the intestinal transit time dose- dependently (Table 4). The 400 mg/kg HELH produced a reduction comparable to that of atropine used here as reference drug and which is known to reduce intestinal motility (Gandhimathi *et al.,* 2009). Since the extract has demonstrated the ability to inhibit castor oil-induced diarrhea, its anti-diarrheic effect might in part be due to decreased gastrointestinal secretion and/or inhibition of gastrointestinal motility. The decreased intestinal motility and intestinal charcoal transit time might be due to increased re-absorption of water as earlier reported by Sahoo *et al*. (2014).

A significant ($p<0.05$) and dose- dependent reduction of castor oil-induced enteropooling was also produced by the extract (Table 5). This observation might be due to the ability of the extract to mediate a reduction in weight gain of intestinal contents by preventing fluid and electrolyte secretion into the intestine through the reduction of gastrointestinal motility (Idakwoji *et al.,* 2018). This is because reduction of the gastrointestinal motility normally allows intestinal content ample time to be exposed to the absorptive surface of the intestinal tract (Friedman *et al.,* 1998).

Diphenoxylate hydrochloride, an opioid, is known to inhibit gastrointestinal secretions and motility, as exhibited by the study extract. Therefore, it could be inferred from the study that the decrease in frequency of defecation and distance travelled by the charcoal meal might be due to the inhibition of the gastrointestinal motility by the extract. It can also be suggested that effects of the extract might be mediated through α-2 adrenergic receptor stimulation.

Antibacterial screening of the extract showed a concentration dependent growth inhibition for both *E. coli* and *Staphylococcus aureus* (Table 6). The zones of inhibition remained clear without bacterial re-growth after 72 hours. One possible mechanism of action is a phytochemical component of the extract such as lectin binding to *E. coli* thereby preventing adhesion to intestinal walls.

It is therefore concluded that the mechanisms by which the leaf extract of *L. hastata* exerts its antidiarrheal effect are by reduction of gastrointestinal motility, anti-secretory and anti-microbial actions which are attributable to the detected phytochemicals. This study also confirms the basis for the ethnobotanical use of the plant for the treatment of diarrhoea.

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