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Full Length Research Paper

Evaluation of the antidiarrhoeal effect of *Lannea welwitschii* Hiern (Anacardiaceae) bark extract

Amole Olufemi Olatokunboh[1]*, Salahdeen Hussein Mofomosara[2] and Onyeahialam Anthony Ekene[3]

[1]Department of Pharmacology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.
[2]Department of Physiology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.
[3]Department of Pharmacology, University of Lagos, College of Medicine Idi-Araba, Lagos, Nigeria.

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Ethnopharmacological relevance encompasses the aqueous bark extract of *Lannea welwitschii* (Hiern), LW is used in Traditional African Medicine (TAM) for the treatment of diarrhea. However, the scientific basis for this usage has not been established. To evaluate the antidiarrhoel activity of LW using various pharmacological methods. The intestinal transit, castor oil induced diarrhea, enteropooling and gastric emptying methods were used in this study. LW (50 - 400 mg/kg per oral (p.o)) produced significant ($P < 0.05$) dose dependant reduction in propulsive movement in both the normal and castor oil induced intestinal transit tests in mice. Peak effect was elicited at 200 mg/kg but this effect was lower than that produced by morphine (10 mg/kg, s.c). The effect of LW on castor oil induced intestinal transit was antagonized by isosorbide dinitrate, IDN (150 mg/kg, P.O.), but not by yohimbine (1 mg/kg s.c.) LW produced a significant decrease in the frequency of defecation, severity of diarrhea and protection from diarrhea in mice treated with castor oil. Also, LW at the dose of 400 mg/kg, significantly ($P < 0.05$) inhibited the castor oil induced intraluminal fluid content. The acute toxicity tests carried out showed a well tolerated effect of the drug via the oral route, a dose of 20 g/kg produced no death in the animals. The LD_{50} was 631 mg/kg given i.p. Phytochemical analysis revealed the presence of alkaloids, saponins, tannins, anthraquinones and reducing sugars. The results obtained in this study suggest that the aqueous bark extract of *L. welwitschii* possesses antidiarrhoel property due to inhibition of gastrointestinal propulsion and fluid secretion possibly mediated through inhibition of the nitric oxide pathway. This justifies the use of the plant extract in TAM for the treatment of diarrhea.

Key words:*Lannea welwitschii,* diarrhea, antidiarrhoeal activity, intestinal transit, enteropooling, gastric emptying.

INTRODUCTION

The use of plants and herbs in curing diseases has always been part of human culture and has transcended all social, economic, religious and other barriers created by man. Plants were the main source of physiologically active substances that were exploited for therapeutic purposes before the advent of synthetic organic chemistry in the nineteenth century. In recent years, there has been renewed interest in the use and efficacy of medicinal plants as a means of not only alleviating, but also treating specific conditions or illnesses.

*Lannea welwitschii (*Anacardiaceae*)* is a tree of about 30m high found in the forests of West Africa. It is found in different parts of Nigeria and ascribed local names include ekika (Yoruba), ewinwan (Edo). In Traditional African Medicine (TAM), *L. welwitschii* is used for swellings, oedema, gout, hemorrhoids and emesis (Iwu, 1993).

Diarrhea is a common gastrointestinal disorder, characterized by an increase in stool frequency and a change in stool consistency (Farthings, 2002). Acute diarrhea is the prevalent form of the disease which has a major impact on the morbidity and mortality in all age groups, particularly in infants and children under the age of three

*Corresponding author. E-mail: femiamole@yahoo.co.uk. Tel: 2348033327658.

(Farthing, 2002; Muriithi, 1996; Hirschhorn, 1980).

In view of these facts, this study was conducted to investigate the antidiarrhoeal activity of the aqueous bark extract of *L. welwitschii*. No report of such study was found in the course of literature study.

MATERIALS AND METHODS

Plant material

The plant material was collected from a farm in Oshogbo, Osun State, Nigeria. Botanical identification and authentication was done by Mr. T. K. Odewo, Senior Superintendent of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen, with identification number FHI 107973, was deposited in the herbarium of the institute.

Preparation of plant extract

200 g of the dried bark was washed and chopped into pieces. The plant material was boiled with 2 L of distilled water in a conical flask for 3 h. The liquid was decanted after 24 h. The filtrate obtained was poured into different beakers of known weight and oven dried at 40°C. The plant residues obtained were then separated and air-dried. After drying, their respective weights were recorded. The stock solution (100 mg/ml) of the dried extract obtained from the plant was prepared and stored in the refrigerator at 4°C. Required concentrations were made from it just before the experiment.

Experimental animals

Albino mice (15 - 30 g) and rats (120 – 200 g) of either sex obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria were used for this experiment. The animals were fed with rodents chow and had free access to drinking water *ad libitum*. All experiments were performed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as contained in United States National Institutes for Health Guidelines (1985).

Acute toxicity test

Groups of mice of both sexes (5 per group) fasted for 12h prior to the test were given LW at doses of 0.1, 1, 5, 10 and 20 g/kg a different set of animals received the extract at doses of 100, 200, 400, 800 and 1600 mg/kg intraperitoneally. Animals in each group were observed for any immediate signs of toxicity and mortality within 24 h. The LD 50 was estimated by the Logdose-probit analysis (Litchfield and Wilcoxom, 1949; Adeyemi et al., 2008; Adeyemi et al., 2009).

Normal intestinal transit

This test was conducted according to the method of Hsu (1982). Mice were allotted to groups of 5 animals each. Treatment was then carried out as outlined below:

Group 1: Distilled water, 10 ml/kg per oral (p.o.)
Group 2: LW 50 mg/kg p.o.
Group 3: LW 100 mg/kg p.o.
Group 4: LW 200 mg/kg p.o.
Group 5: LW 400 mg/kg p.o.
Group 6: Morphine, 10 mg/kg s.c.

30 min after, charcoal meal (10% charcoal in 5% gum acacia; 0.2 ml/mouse, p.o) was administered to each animal. The mice were sacrificed 30 min later and the small intestine was immediately isolated. The peristaltic index (PI) which is the distance traveled by the charcoal meal relative to the total length of small intestine expressed in percentage was determined for each mouse (Aye-Than et al., 1989; Adeyemi et al., 2009).

Castor oil induced intestinal transit

The same procedures as in the normal intestinal transit test were followed except that castor oil (0.2 ml/mouse, p.o.) was administered 30 min before administration of charcoal meal that is, 30 min post treatment (Hsu, 1982; Aye-Than et al., 1989). Additional groups comprised sets of animals that received yohimbine (1 mg/kg, s.c.; group 7) and isosorbide dinitrate, IDN (150 mg/kg, s.c; Group 8) 15 min before LW (200 mg/kg; p.o.)

Castor oil induced diarrhea

Groups of 15 mice each were treated as outlined below:

Group 1: Distilled water, 10 ml/kg p.o.
Group 2: LW 50 mg/kg p.o.
Group 3: LW 100 mg/kg p.o.
Group 4: LW 200 mg/kg p.o.
Group 5: LW 400 mg/kg p.o.
Group 6: Morphine, 10 mg/kg s.c.
Group 7: IDN 150 mg/kg p.o (given 30 min prior to the administration of LW 200 mg/kg p.o)

Thirty minutes after castor oil (0.2 ml/mouse) was administered to each mouse. The animals were then placed under separate glass funnels with the floor lined with blotting paper for observation for 4 h (Izzo et al., 1992). The following parameters were observed: onset of diarrhea, number of wet faeces and total weight of faecal output.

Intestinal fluid accumulation

Following the method of Robert et al. (1976), rats divided into 5 animals per group were pretreated with distilled water (10 ml/kg p.o) and LW (100 and 400 mg/kg p.o). One hour after, the rats received castor oil (2 ml/rat) intragastrically. The animals were killed 1 h later and the small intestines were removed after ligation at the pyloric end and ileocaecal junction respectively and weighed. The contents of the intestine were then expelled into a graduated tube and the volume measured. The intestines were reweighed and the differences between the full and empty intestines were calculated.

Gastric emptying

Rats fasted for 24 h were randomly allotted to two groups of five animals each. The different groups received distilled water (10 ml/kg p.o); LW (400 mg/kg per oral). One hour later, 3 ml of a semi-solid meal based on methylcellulose was administered to the animals. The rats were sacrificed and laparatomized 1 h after with the stomachs removed.

The full stomachs were weighed, opened and rinsed. The empty stomachs were reweighed. The difference in weight between the full and empty stomachs was subtracted from the weight of 3 ml of the test meal (Droppleman et al., 1980).

Table 1. Effect of *Lannea welwitschii* on normal intestinal transit in mice.

Group	Dose (mg/kg)	Peristaltic index %	inhibition%
Control	-	73.97 ± 0.15	-
LW	50	57.12 ± 1.42*	23.10
LW	100	54.28 ± 2.44*	26.72
LW	200	52.20 ± 3.40*	29.58
LW	400	65.04 ± 2.69	33.09
Morphine	10	36.76 ± 2.69*	50.30

Values are mean ± S.E.M (n = 5) P < 0.05 vs control (student's t-test).

Table 2. Effect of *Lannea welwitschii* on castor oil induced intestinal transit in mice.

Group	Dose(mg/kg)	Peristaltic index %	inhibition %
Control	-	81.57 ± 3.18	-
LW	50	42.28 ± 3.02*	48.17
LW	100	38.67 ± 3.52*	52.60
LW	200	34.63 ± 3.42*	58.55
LW	400	44.55 ± 2.11*	78.88
LW+	200	32.62 ± 4.53	60
Yohimbine	1		
LW+	200	49.92 ± 3.18*	38.8
Isosorbide	150		
Morphine	10	18.15 ± 1.52*	77.88

Values are mean ± S.E.M (n=5) P < 0.05 vs control (student's t-test).

Phytochemical analysis

Preliminary phytochemical analysis of the plant extract was carried out according to the methods of Odebiyi and Sofowora (1978).

Drugs

Castor oil (Finest Cold Drawn Commercials castor oil), Isosorbide dinitrate, yohimbine (Sigma Chemical, Company USA), morphine (Evans medical Ltd; Liverpool), and Methylcellulose (Koch-Light Laboratories Ltd; England).

Data analysis

Results obtained from the study were expressed as mean + SEM. Statistical analysis of the data was done using students t-test or chi-squared test, where appropriate. Results were considered significant when P < 0.05.

RESULTS

Acute toxicity test

Oral administration of the aqueous bark extract of LW in doses up to 20 g/kg did not produce any mortality and visible signs of toxicity when observed for 2 h after treatment and for further 7 days. Administered intraperitoneally, the extract produced visible signs of toxicity in mice at doses of 800 and 1600 mg/kg. These include abnormal gait, increased respiration, decreased activity, writhing and piloerection, the extract shows dose dependent mortality. The LD_{50} was estimated to be 631 mg/kg for the intraperitoneal route.

Normal intestinal transit

In control animals, the charcoal meal traversed 73.97 ± 0.15 of the total length of the small intestine. The aqueous bark extract of *L. welwitschii,* LW (50 – 400 mg/kg) produced significant (P < 0.05) dose dependant reduction in normal intestinal transit. Peak effect was produced at 400 mg/kg, giving a peristaltic index of 65.04 ± 2.69 corresponding to 33.09% inhibition. This effect was lower than that elicited by morphine (10 mg/kg s.c) which gave a peristaltic index of 36.76 ± 2.69 corresponding to 50.30% inhibition (Table 1).

Castor oil induced intestinal transit

Table 2 shows the effect of castor oil on intestinal transit in mice. The extract (50 – 400 mg/kg) cause a significantly (P < 0.05) dose dependent reduction in the

Table 3. Effect of *Lannea welwitschii* on castor oil induced diarrhea in mice.

Group	Dose(mg/kg)	Onset of diarrhoel (min)	No. of wet stool	Total No of stool	Weight of wet stool	Total weight of stool	Diarrhoea score	Protection %
Control	-	66.5 ± 3.64	8.50 ± 1.06	10.0 ± 1.18	0.29 ± 0.06	0.32±0.07	25.3±3.37	-
LW	50	64.5 ± 9.60	6.67 ± 0.56	9.17 ± 1.17	0.26 ± 0.06	0.30±0.06	18.5±1.31	26.90
LW	100	82.7 ± 1.77	3.67 ± 0.07*	5.83 ± 1.18*	0.17 ± 0.03	0.21±0.04	11.7±1.22*	53.93
LW	200	169 ± 23.1*	2.50 ± 0.72*	6.00 ± 1.29*	0.13 ± 0.05*	0.21±0.06	9.83±2.1*	61.19
LW	400	115 ± 5.52*	4.83 ± 0.40*	10.0 ± 1.29	0.28 ± 0.08	0.37±0.10	17.2±1.7*	63.25
LW	400+150	136 ± 21.5*	4.00 ± 0.68	14.8 ± 1.53*	0.23 ± 0.05	0.41±0.07	20.2±2.01	20.37
Morphine	10	169 ± 13.2*	2.33 ± 0.88*	2.50 ± 0.92*	0.13 ± 0.06	0.13±0.06	5.83±21.0*	76.98

Values are mean ± S.E.M (n=5); P < 0.05 vs. control (student's t-test). Diarrhoeal score was analyzed by Chi's square test. *P < 0.05 vs control.

Table 4. Effect of *Lannea welwitschii* on intestinal fluid accumulation in rats.

Group	Dose (mg/kg)	weight of intestinal content (g)	Volume of intestinal content (ml)
Control	-	2.57 ± 0.18	2.33 ± 0.17
LW	400	1.58 ± 0.32*	1.40 ± 0.25*

Values are mean ± S.E.M (n=5); P<0.05 vs. control.

Table 5. Effect of *Lannea welwitschii* on gastric emptying in rats.

Group emptied	Dose (mg/kg)	Difference btw weight of full empty stomach (g)	Quantity (g)
Control	-	1.67 ± 0.28	1.38 ± 0.26
LW	400	1.47± 0.13	1.62 ± 0.15

Values are mean ± S.E.M (n = 5)

distance traveled by the charcoal meal compared to the control mice. The highest inhibition of 58.55% of normal intestinal transit was produced by the extract at 200 mg/kg and this was significantly ($p < 0.05$) less than the effect 77.88 produced by morphine (10 mg/kg). The effect of the extract was not antagonized by yohimbine (1 mg/kg) but it was antagonized by isosorbide dinitrate (150 mg/kg).

Castor oil induced diarrhea

In the course of observation for 4 h after castor oil administration, all the mice in the control group (distilled water 10 ml/kg p.o.) produced copious diarrhea. Pretreatment of mice with the aqueous bark extract of LW (50 – 400 mg/kg) caused a significant ($P < 0.05$) dose dependent delay in the onset of copious diarrhea, decreased the frequency of purging (reduction of number of wet stools and total no of stools), weight of wet stools and severity of diarrhea (general diarrhea score). Peak effect was produced by the extract at 400 mg/kg, but this was significantly ($p < 0.05$) less than the effect produced by morphine. Isosorbide dinitrate (IDN) significantly inhibited the onset of diarrhea in mice and other parameters such as number of wet stools, total number of stools and the diarrhea score. The protection produced by the extract (400 mg/kg) on mice as measured using the diarrhea score was decreased (by about 40%) by IDN administered 30 min before the extract. Results are as shown in Table 3.

Intestinal fluid accumulation

Oral administration of castor oil produced intestinal fluid volume of 2.33 ± 0.17 ml, the aqueous bark extract of LW at 400 mg/kg significantly ($p < 0.05$) reduced the volume of intestinal fluid to 1.40 ± 0.25 (Table 4).

Gastric emptying

As shown in Table 5, the aqueous bark extract of LW did not produce any significant effect on gastric emptying.

Phytochemical analysis

This revealed the presence of oils, reducing sugars, alkaloids, saponins, tannins and anthraquinones in the

aqueous bark extract of *L. welwitschii.*

DISCUSSION

The phytochemical analysis of the aqueous bark extract of *L. welwitschii* showed the presence of bioactive compounds such as saponins, tannins, alkaloids and anthraquinones. Some of these compounds have been shown to inhibit intestinal mobility in a dose related manner (Carlo et al., 1994). Tannins are best known to decrease the irritability of the bowel thereby reducing peristaltic index (Oliver, 1960). The extract produced a decrease in propulsive movement at the standard charcoal meal in the small intestine, suggesting an antispasmodic activity. This activity was dose dependent with the greatest effect shown at 400 mg/kg of the extract. The extract was more effective in the castor oil induced intestinal transit than in the normal transit, this result suggested that the extract may be more effective in a deceased state than in normal state. Results of this study shows that the α-2adrenergic antagonist yohimbine did not influence the action of the extract on intestinal propulsion, but the nitric oxide donor, isosorbide dinitrate, IDN has influence on the action of the extract on intestinal propulsion. This suggests a lack of α-2adrenoceptor stimulant effect of the extract and the presence of nitric oxide inhibitory effect of the extract. The reduction of the intestinal transit following administration of the extract before and after the onset of castor oil induced diarrhea showed the ability of the extract to prevent diarrhea as well as an ability to suppress already established diarrhea respectively. Castor oil after administration usually cause the release of ricinoleic acid and this usually causes a change in the integrity of the fluid and electrolyte balance in the mucosa of the gastrointestinal tract. LW (400 mg/kg) significantly ($p < 0.05$) inhibited the castor oil induced intestinal fluid accumulation (entero-pooling) with little changes in the weight of the intestinal content. LW produced a dose dependent reduction in the frequency and severity of diarrhea produced by castor oil. There was delay in the onset of diarrhea with protection of about 62% at 200 mg/kg which is the most effective dose. The total number of stools, total weight of stools and weight of wet stools were all decreased in a dose dependent manner with the highest effect observed at 400 mg/kg of the extract. However, the effects of the extract on these diarrhea parameters were significantly less than those produced by morphine. The general diarrhea score of the extract (400 mg/kg) was 63.25% compared to morphine (10 mg/kg, 76.98%).

Administration of the extract, orally in divided doses up to 20 g/kg shared no mortalities; however, the intra-peritoneal route produced toxic effects with LD_{50} of 631 mg/kg body weight. This result shows that the aqueous extract is better tolerated when administered orally than the intra-peritoneal route. Thus, it is relatively safe through the oral route.

Conclusion

From the results obtained, it can be concluded that the aqueous bark extract of *L. welwitschii* produced an inhibitory action on gastrointestinal mobidity and secretion and the mechanism may be *via* the inhibition of nitric oxide. Further tests needed to be done to determine the active component responsible for the antidiarrhoel activity of the extract.

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