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Phytochemical and Antidiarrhea Activity of the Methanolic Extract of the Stem Bark of *Lannea kerstingii* Engl. and K. Krause (Anacardiaceae)

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ABSTRACT

Lannea kerstingii Engl. and K. Krause is used in Nigeria and Northern Côte d'Ivoire for the management of diarrhea. Crude methanolic extract of stem bark of Lannea kerstingii was evaluated on isolated rabbit jejunum and castor oil-induced diarrhea model in rats. The methanolic extract of Lannea kerstingii (1, 10 and 100 mg/ml) produced a dose dependent transient contractile response followed by relaxation of isolated rabbit jejunum. In the castor oil-induced diarrhea model, 60.0% protections was produced by the extract. These results revealed that the methanolic extract of the stem bark of Lannea kerstingii (300 and 600 mg/Kg) have antidiarrhea activity. The effects on smooth muscle showed the usefulness of the Lannea kerstingii in the management of diarrhea. The effect of Lannea kerstingii was not as comparable to that of loperamide, a strong antidiarrhea drug, in castor oil induced diarrhea. This may partially explain the use in traditional medicine for the treatment of diarrhea. These results support their use in folk medicine.

Key words: lannea kerstingii, antidiarrhea, diarrhea, castor oil

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INTRODUCTION

Diarrhea, which is the passage of abnormal liquid or unformed stool at an increased frequency can be caused by infectious agents, certain medications, plant and animal toxins, gastro-intestinal disorders, and substances that increase gastrointestinal tract secretions. It can also be caused by the ingestion of poorly absorbable materials, or inflammatory and dysmotility problems of the gastro-intestinal tract [1].

Annually, diarrhea diseases causes several million of deaths in the world [2]. It is the most common causes of morbidity and mortality in developing countries [3]. Infants younger than 1 year account for more than half of these deaths, and the risk can be 2 - 3 times higher among infants who are not exclusively breast-fed [4].

In Nigeria, diarrhea infection remains the number one killer disease among children under 5 years, while 7 – 12 months old babies are the most susceptible [5].

With over half of Nigerian population living in poverty [6], majority of its population often use medicinal herbs for the management of diarrhea and other related health problems. Some herbal medicines (e.g. *Xylocarpus granatum*

and *Guiera senegalensis*) have been reported to be scientifically effective in the management of diarrhea. Some medicinal practitioners in Nigeria use *Lannea kerstingii* Engl. and K. Krause for the treatment of diarrhea thus it is from this base that the antidiarrhea activity of this plant is investigated. The stem bark and roots of *Lannea kerstingii* are consumed by natives from Northern Côte d'Ivoire, as traditional remedies for the treatment of diarrhoea, gastritis, rheumatic, sterility, intestinal helminthiasis [7].

Lannea kerstingii Engl. and K. Krause is a tree with a height of 12 m, usually highly branched and distributed throughout the Sudanese and Guinean Savannah [8].

MATERIALS AND METHODS

Collection of Plant materials: *Lannea kerstingii* was collected from Samaru-Zaria in the month of May, 2011 and was authenticated at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria-Nigeria by a botanist (A.U. Gallah) where a voucher specimen (1832) was deposited for future references. After identification, stem bark was removed and dried under shad. When dried, the size was reduced using mortar and pestle, filtered for homogeneity and kept for further use.

Preparation of plant materials: About 1kg of *Lannea kerstingii* was put in an extraction bottle and 1.5 liters of methanol poured into it and allowed to soak for 4 days where the solvent was filtered and replaced every 24 hours. The filtrate was then transferred to an evaporating dish and was evaporated using a water bath (35^{o}C) to obtain a brown mass (methanolic extract). Solutions of the extracts were prepared freshly for each study.

Animals: New Zealand rabbit (*Oryctolagus cuniculus*) weighing 800g and Swiss albino mice (16-30g) of both sexes were maintained in the Animal House Facility of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria, Nigeria. The animals were fed with standard laboratory feeds (Vital Feeds, Nigeria) and tap water (*ad libitum*).

This research was carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally.

Drugs: Acetylcholine (Sigma chemical, USA), Castor oil (Bell Sons and Co., England) and Loperamide (Janssen)

Phytochemical procedure: The preliminary phytochemical screening of the crude extract of *Lannea kerstingii* was carried out in order to ascertain the presence of its constituents by utilizing standard conventional protocols [9].

Acute Toxicity Study: The method described by Lorke [10] was adopted using13 mice. In the first phase, three doses of the methanolic extract (10, 100 and 1000mg/kg were administered to three groups each containing three mice). In the second phase, more specific doses were administered to four groups each containing one mouse. The median lethal dose (LD_{50}) was determined as the geometric mean of the highest non lethal dose and lowest lethal dose of which there is 0/3 and 0/1 survival.

Effects on isolated rabbit ileum: The rabbit was made unconscious by a powerful strike at the back of the neck (stunning). The abdomen was immediately opened using forceps and part of the ileum was quickly removed. The ileum was introduced immediately into a Petri dish of saline containing Tyrode solution (NaCl, 136.8; KCl, 2.7; CaCl, 1.3; $NaHCO_3$, 12.0; MgCl, 0.5; $NaPO_4$, 0.14; glucose, 5.5. millimole) and each end of the ileum was tied with a thread.

The tissue was mounted in a 50 ml organ bath containing Tyrode.s solution maintained at 37^{o}C and aerated with air. A load of 0.5 g was applied. Equilibration period of 60 minute was allowed during which the physiological solution was changed at every 15 min. One end of the ileum was attached to the transducer which measured the mechanical impulse of the tissue and converted it to electrical impulses which was then recorded on microdynamometer. At the end of the equilibration period, the effects of Acetylcholine ($1x10^{-5}$ g/ml), and the methanolic extract were determined.
Subsequently, solutions of acetylcholine and methanolic extract of the stem bark of *Lannea kerstingii* were added at intervals to the isolated perfuse chamber. After application of each drug, the tissue was washed three times with the Tyrode solution to remove every trace of the drug [11].

Effects of castor oil induced diarrhea in mice: The mice were fasted for 12 hours prior to the commencement of the experiment and were randomly divided into five groups of five mice each. The mice in the first group received 10mlkg^{-1} normal saline intraperitoneally while the mice in the second received 5mlkg^{-1} Loperamide as a standard positive control, the third, fourth and fifth 150, 300 and 600mgkg^{-1}, respectively. After 30 minutes of administration of the extract, castor oil 0.2ml/mouse were administered orally [12]. The animals were placed on individual special cages over white clean whatman filter.

Statistical analysis: The results were analysed by chi square (X^2). Values were considered significant with P< 0.05 for both isolated tissue and castor oil induced diarrhea.

RESULTS

Phytochemical analysis: The preliminary phytochemical screening of the extract revealed the presence of tannins, flavonoids, alkaloids, anthraquinones and steroids.

Acute Toxicity study: The median lethal dose of the extract was found to be greater than 2154mg/kg bodyweight.

Table 1. Effect of Crude Methanolic Extract of *Lannea kirstingii* on Castor Oil Induced Diarrhea in Mice

Treatment (mg/kg)	Dose (mg/kg)	No of mice With Diarrhea	% protection
Normal saline	10ml/kg	5/5	0.0
Extract	150	3/5	40
Extract	300	2/5	60
Extract	600	2/5	60
Loperamide	5	0/5	100

Results were analyzed by Chi-square (X^2). Values were considered significant when P<0.05 compared with Normal saline group, n=5

Table 1 shows that the extract was dose dependent since at 150mg/kg, the protection was 40% and when the dose increased to 300 and 600 mg/kg, the percentage protection increases to each 60% respectively. Though it was not as comparable to loperamide whose protection was 100%.

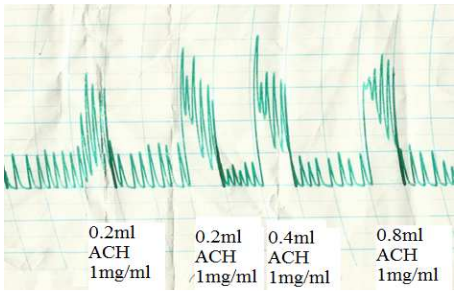

Figure 1 Effects of Acetylcholine (ug/ml) on isolated rabbit ileum

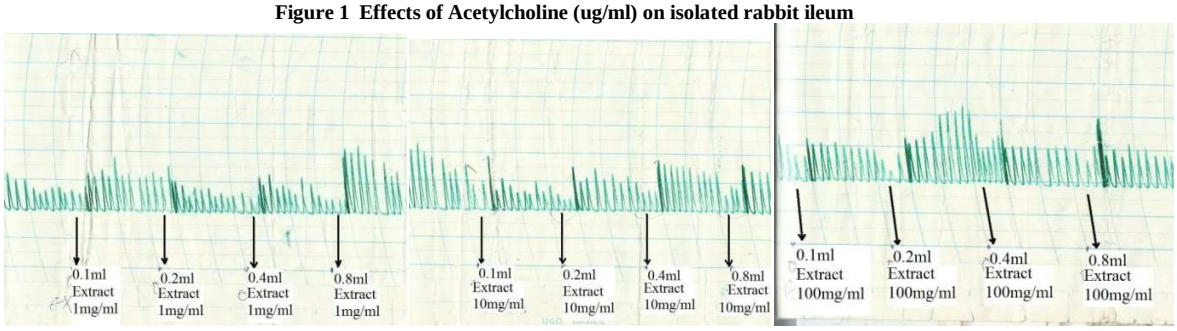

Figure 2. Effect of methanolic extract (1, 10 and 100mg/ml) of the stem bark *Lannea kerstingii* on isolated rabbit ileum

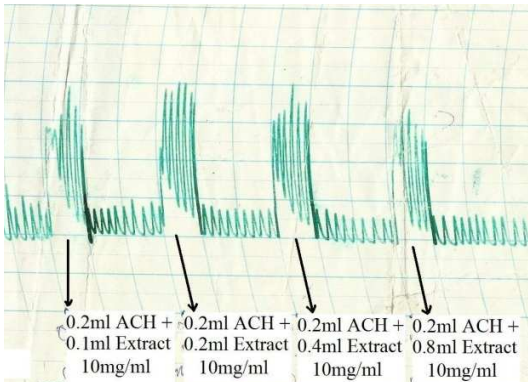

Figure 3. Effect of methanolic extract of the stem bark *Lannea kerstingii* and Acetylcholine on isolated rabbit ileum

DISCUSSION

Phytochemical analysis: The preliminary phytochemical screening of the extract revealed the presence of alkaloids, saponins, flavonoids, tannins and steroids.

Acute Toxicity study: The median lethal dose of the extract was found to be 2154.1mg/kg bodyweight.

Effect of methanolic extract of *Lannea kerstingii* on isolated rabbit ileum:
Acetylcholine cause increased contraction of the isolated jejunum as shown in Fig.1. However, *Lannea kerstingii* significantly reduced intestinal transit time as observed by the decrease in intestinal motility of isolated rabbit jejunum as shown in figure 2. It also revealed that the effects of the extract on the isolated rabbit ileum were dose related and the extract relaxed the spontaneous contraction of the rabbit ileum (figure 2).

The effects of both the extract and acetylcholine still shows contraction as can be seen in fig. 3 which suggest that the extract act in a different route from acetylcholine [13].

Effect of *Lannea kerstingii* on castor oil induced diarrhea
The extract produced a dose dependent protection against the castor oil- induced diarrhea with the highest protection (60%) obtained at the dose tested (300mg/kg and 600mg/kg) though not as comparable to that of loperamide (100%), the standard anti diarrhoeal agent (Table 1).

Castor oil causes diarrhea due to its active metabolite, ricinoleic acid [14, 15] which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin [11].

Acetylcholine (ACH) cause increased contraction of the isolated jejunum as shown in Figure 1. However, *Lannea kerstingii* significantly reduced intestinal transit time as observed by the decrease in intestinal motility of isolated rabbit jejunum as shown in Figure 2.

Phytochemical screening revealed the presence of alkaloids, tannins and sterols. Earlier studies showed that antidiarrhea properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids and sterols [11, 16]. Hence, tannins, sterols, alkaloids may be responsible for the mechanism of action of *Lannea kerstingii* anti-diarrhea activity. The anti-diarrhea activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistance and hence, reduce secretion. This can be due to the fact that the extract increased the reabsorption by decreasing intestinal motility in isolated rabbit ileum.

Mice administered with 150, 300 and 600mg/kg methanolic extract of *Lannea kerstingii* had diarrhea in 3/5, 2/5 and 2/5 respectively (40, 60, and 60% protection respectively) as shown in Table 1. In this study, methanolic extract of *Lannea kerstingii* exhibited anti-diarrheal activity. Its effect was dose dependent. The results were not as significant as that of the standard drug Loperamide 5mg/kg with regard to the severity of diarrhea. The observed relaxation

exhibited by the stem bark extract further explains its ability to protect the mice against diarrhea induced by castor oil.

CONCLUSION

The methanolic extract of the stem bark of *L. kerstingii* was found to contain alkaloids, saponins, flavonoids, tannins and steroids. The extract at a dose of 1mg/ml, 10mg/ml and 100 mg/ml produced a dose dependent transient contractile response followed by relaxation of isolated rabbit jejunum. In the castor oil-induced diarrhea model, 60.0% protections was produced by the extract. These results revealed that the methanolic extract of the stem bark of *Lannea kerstingii* at a dose of 300mg/kg and 600 mg/Kg have antidiarrhea activity. This confirms the use of this part of the plant in the management of diarrhea.

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