



SPASMOLYTIC AND ANTI-DIARRHEA EFFECTS OF THE BARK OF *ERYTHRINA SENEGALENSIS* AND ROOT OF *KIGELIA AFRICANA*

OTIMENYIN S O* AND UZOUCHUKWU D C

*Departments of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Plateau State, Nigeria.
E mail: sundayo@unijos.edu.ng

ABSTRACT

Erythrina senegalensis and *Kigelia Africana* are used for the management of chronic abdominal pain in Northern Nigeria. Anti-diarrhea activities of these plants was evaluated *in-vivo* using castor oil induces diarrhea model in rats and *in-vitro* using isolated rabbit jejunum. *E. senegalensis* (500 and 1000 mg/kg respectively) and *K. africana* (500 and 1000 mg/kg) significantly ($P < 0.05$) reduced the frequency of diarrhea stool and the spontaneous propulsive movement of isolated rabbit jejunum (anti-motility). *E. senegalensis* extract irreversibly inhibited Acetylcholine (Ach) induced motility of rabbit jejunum, while *K. africana* extract produced reversible inhibition of Ach induced motility of isolated rabbit jejunum. The observed spasmolytic effects of these extracts (*E. senegalensis* and *K. africana*) may explain their continual use in the management of chronic abdominal pain and pains associated with diarrhea. This study revealed that the extract also possesses anti-diarrhea activity for which the plant is not used traditionally.

Key words: *Erythrina senegalensis*, *Kigelia Africana*, Anti-diarrhea activity, Spasmolytic effects.

INTRODUCTION

In African herbal medicine, herbs are used for the management of disease conditions, including diarrhea and abdominal pain. Diarrhea presents as a disease or as symptoms of some disease conditions. It is associated with viral, bacteria and fungi infection, food poisoning and other disease conditions. Uncontrolled diarrhea is dangerous as it can lead to loss of body fluid and electrolyte imbalance. Excessive loss of body fluid results in severe dehydration and death. Maintenance of electrolyte balance and replacement of fluid is therefore crucial in the management of diarrhea. Children with diarrhea are often at risk, as little loss of body fluid can be fatal. Search for potent and cheap drug with anti-diarrhea properties is on with the hope of discovering well tolerated anti-diarrhea drug with fewer side effects. Presently, the management of diarrhea involves the use of anti-motility agents, anti-secretory agents, anti-fungi and anti-bacteria agents and /or oral rehydration therapy (ORS). Low income earners or jobless Nigerians depend on herbal medicines to meet their health needs. Evaluation of the pharmacological properties of such herbs is imperative as it will enable health workers to advice patients appropriately.

Some pharmacologically active plants have been confirmed to have anti-diarrhea properties. They include; *occimum grattissimum*¹, *Asparagus pubesons*, *Conyza dioscoridis*, *Alhagi maurorum*, *Mentha microphylla*, *zygophyllum album* and *Conyza linifolia*² *Pergularia daemia*³ and *Moringa oleifera*⁴.

K. africana (Lam) Benth, is used locally in Nigeria for the management of chronic abdominal pain. It is commonly called Sausage tree (English); Worsboom (Africa); Muvevha (Venda); um vungata, umfongothi (zulu); Rawaya (Hausa). *K. africana*, is found in tropical Africa (Eritrea, Chad, South Africa, Senegal, Nigeria and Namibia). In the United States, *K. africana* is one of the active principles in a skin care product (www.sheatteraorganics.com). Its fruits are prepared for consumption by drying, roasting or fermentation^{5,6}. In Botswana the timber is used as yokes and oars⁷.

E. senegalensis a. dc. is a coral tree. Coral trees are used widely in the tropics and subtropics as street and park trees, especially in drier areas. It is found in Mali, Senegal, and Nigeria. *E. senegalensis* is used in traditional medicine for the management of amenorrhoea, dysmenorrhoea, malaria, infection, wound and body pain (chest pain, back pain, abdominal pain), headaches and body weakness, gastric ulcer, diarrhea and constipation⁸. *E. senegalensis* is well studied, chemicals elucidated from it include: Prenylflavonoids, 8-prenylleutone, auriculatin, erysenegalensein O, erysenegalensein D, erysenegalensein N, derrone, alpinumisoflavone, and 6, 8-diprenylgenistein⁹.

The plant also contain isoflavonoid (2, 3 dihydroauriculatin, and 6-8 diprenylgenistein) with anti-bacterial activity¹⁰, flavonoid (Erybraedin A) with anti-microbial and anti-fungal properties, Lonchocarpol A and prenylisoflavonoid with HIV-inhibitory properties,¹¹ and Alpumisoflavone, a substance used for the management of schistosomiasis infection.

MATERIALS AND METHODS

Animals

Albino rats (weighing between 125 - 220g), and Rabbits (1.7 - 1.9Kg) were used for this experiment. They were purchased from the University of Jos, Animal House. They were fed with standard commercial feeds (Vital Feeds, Nigeria) and tap water (*ad libitum*).

Collection and Preparation of Plant Materials

The bark and root of *E. senegalensis* and *K. africana* respectively were collected in the month of September 2008 by a herbalist in Jos, Plateau State, Nigeria. The plants were authenticated by Dr. A. Karim of the Federal School of Forestry, Jos. Collected plant materials were dried under shade to prevent direct effect of sun, which might affect the chemical constituents in the extract. The resultant dried plant parts were individually reduced to powder with mortar and pestle, sieved and kept in a clean dried cupboard before use. Ninety grams of each of the powdered plant materials were separately soxhlet extracted for 72 hours with ethanol at 50°C. The resultant dried extracts were labeled and preserved in the refrigerator until needed.

Castor oil Induced Diarrhea in Rats

The method described by Galvez¹² was used for this experiment. Male and female wistar rats (weighing 125-220g) were divided into six groups of five animals each and housed in separate cages, during screening. The rats were denied access to food for 24 hours before commencement of the experimental procedures, but allowed free access to water. Animals in group one received 0.5ml of distilled water (intra-peritoneally, IP) while animals in group two received loperamide (4 mg/kg). Groups three and four received methanolic extract of *K. africana* (500 mg/kg and 1000 mg/kg respectively), IP, while animals in groups 5 and 6 rats received methanolic extract of *E. senegalensis* (500 mg/kg and 1000 mg/kg respectively). Thirty minutes after treatments, each animal received 0.5 ml of castor oil orally via orogastric cannula. The animals were then placed in separate cages over clean white paper and observed for 6 hours for the onset of diarrhea, and number of diarrhea episodes. Absence or reduction in number of loose stool was recorded as a protection from diarrhea¹³.

Anti-motility activity

Rabbits were killed by a blow on the head, dislocating the neck, and exsanguination. Segments of the jejunum, about 2.0cm long, were removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode solution. The isolated tissue was mounted in a 50ml organ bath containing tyrode's¹³ solution maintained at 37°C and aerated with air. A load of 0.5g was applied and equilibration period of 60 minutes was allowed during which the physiological solution was changed every 15 minutes. At the end of the equilibration period, the effects of acetylcholine (1×10^{-5} g/ml), were determined. The effect of graded doses of the extracts of bark of *E. senegalensis* (0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 ug/ml) and the extract of the roots of *K. africana* (0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 ug/ml) were recorded using Ugo basile unirecorder 7050. The effects of graded doses of acetylcholine (0.02, 0.04, 0.08, 0.12, 0.16 and 0.2ug/ml) in the presence of graded doses of *E. senegalensis* and *K. africana* were also recorded.

RESULTS

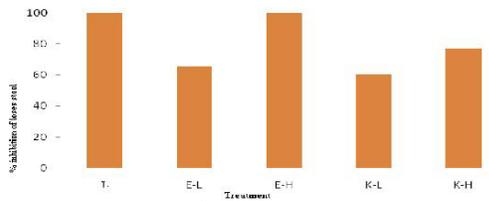


Fig. 1: Percent inhibition of loose stool by extract and drug

Key: L = Loperamide, E.L= 500 mg/kg *E.senegalensis*, E-H= 1000 mg/kg *E.senegalensis*, K-L= 500 mg/kg *K.africana*, K-H= 1000 mg/kg

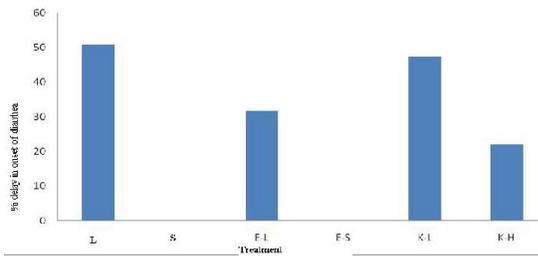


Fig. 2: Percentage delay in onset diarrhea inhibition of loose stool by extract and drug

Key: S- Distilled water, L = Loperamide, E.L= 500 mg/kg *E.senegalensis*, E-S= 1000 mg/kg *E.senegalensis*, K-L= 500 mg/kg *K.africana*, K-H= 1000 mg/kg *K.africana*

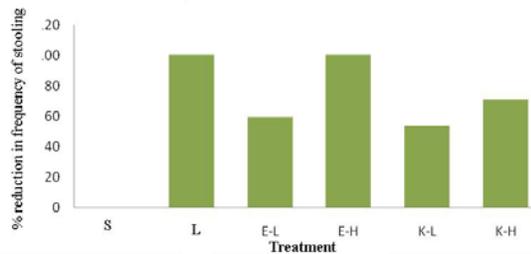


Fig. 3: Percentage reduction in the frequency of stooling

Key: S= Distilled water, L = Loperamide, E.L= 500mg/kg *E.senegalensis*, E-H= 1000 mg/kg *E.senegalensis*, K-L= 500

Table 1: Effects of Methanolic Extracts of *E. senegalensis* and *K. africana* on Castor Oil Induced Diarrhea

Treatment dose	Mean onset of Diarrhea (hrs)	Mean weight of stool (g)	Mean No. of loose stool
Control (0.5ml distilled water)	2.95±1.83	6.73±0.68	4.3±1.77
Loperamide 4mg/kg	-	-	-
<i>E. senegalensis</i> 500mg/kg	4.10±1.62 ^a	2.3±1.16 ^b	1.75±0.83 ^b
<i>E. senegalensis</i> 1000mg/kg	-	-	-
<i>K.africana</i> 500mg/kg	3.17±1.13 ^a	2.65±0.7 ^b	2.00±0.71 ^b
<i>K.africana</i> 1000mg/kg	4/69±0.82 ^a	1.55±0.46 ^b	1.25±0.75 ^b

a= P>0.05, b= P<0.05, when values were compared with control values. Values are in Mean ± Standard Error of Mean (SEM). N = 5

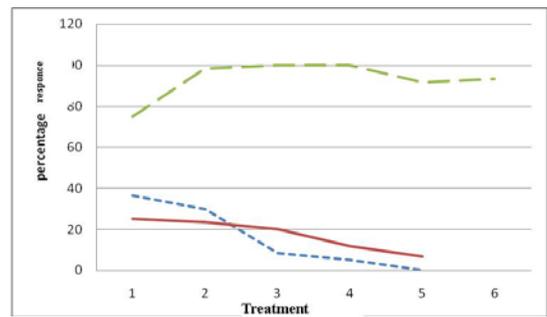


Fig. 4: Effect of ach and extracts on the jejunum

--- = Ach = *K. Africana* _____ = *E.senegalensis*

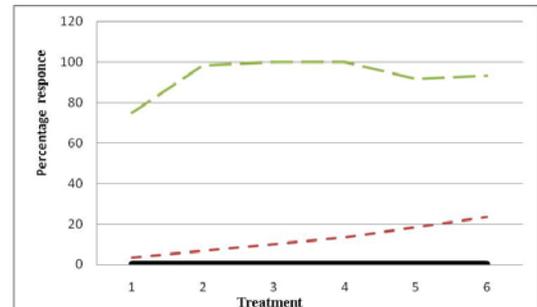


Fig. 5: Effect of ach in the presence of extracts

_____ = *E.senegalensis* + Ach = *K. Africana* + Ach
--- = Ach

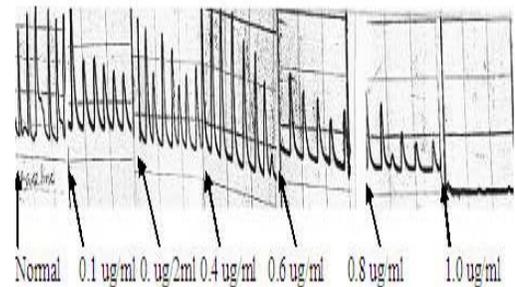


Fig. 6: Effect of *E.senegalensis* on isolated rabbit jejunum

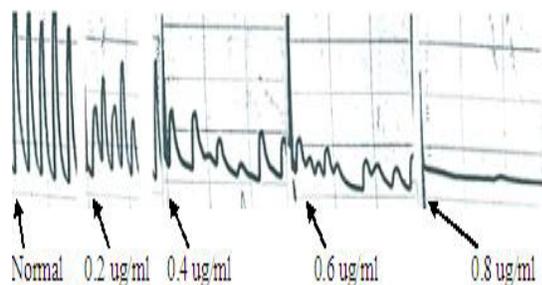


Fig.7: Effect of *K. Africana* on isolated rabbit jejunum

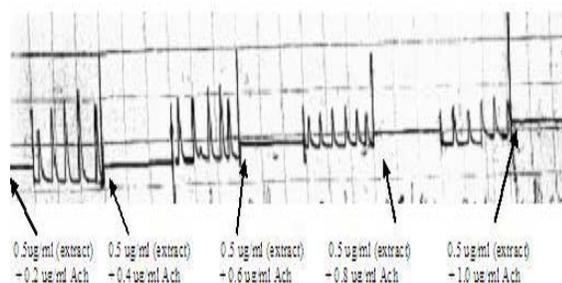


Fig. 8: Effect of Ach in the presence of *E. senegalensis*

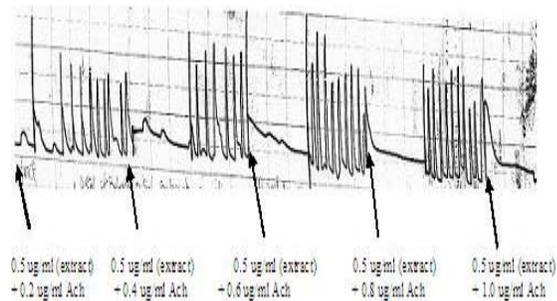


Fig. 9: Effect of Ach in the presence of *K. Africana*

DISCUSSION

Diarrhea is one of the commonest gastrointestinal (GI) disorders in children (in form of acute gastroenteritis) and adults. Diarrhea often presents as a sign of an underlying disease condition such as an infection (from food poisoning) or gastro-intestinal disorders¹⁴, treatment of diarrhea involve the use of anti-infective agents (where microorganisms are implicated), oral rehydration solution (when excess fluid is lost), and non antimicrobial anti-diarrhea agents (such as anti-motility agents, opiates and adsorbents)¹⁵.

However, attention to traditional medicine all over the world is on the increase especially in African nations where there is increasing debt and high cost of modern health care⁴. Low income Nigerians depend on herbs for the management of diarrhea before the advent of orthodox medicine. These set of patients are still using herbs for the management of diarrhea. Some of the plants used, has been evaluated and majority were found to have pharmacological activity (Examples include the root of *Asparagus pubescens* and the bark and resins of *Moringa oleifera*⁴).

The bark of *E. senegalensis* and the root of *K. Africana* are used for the management of colic pain (which often is associated with diarrhea. Results obtained from this study suggest that the methanolic extract of the bark of *E. senegalensis* and the root of *K. africana* possesses antidiarrhea activities. In castor oil induced diarrhea, both extracts dose dependently reduced the onset of diarrhea and amount of watery stool produced by the Prolonged the onset of diarrhea, but significantly ($P < 0.05$) reduced the frequency of loose stool and the

amount of stool produced. Higher dose of the extract produced greater significant reduction in the amount of stool excreted and the amount of loose stool. This implies that the extract dose dependently inhibits the production of loose stool. The anti-diarrhea effects observed was comparable to the anti-diarrhea effect of Loperamide (4 mg/kg), a standard anti-diarrhea drug.

K. africana (500mg/kg) prolonged of the onset of diarrhea (Table 1, Figure 2), reduced the frequency of stooling and inhibited loose stool production (Figure 3). At a higher dose, it produced greater effects, revealing that the extract produces dose dependent anti-diarrhea effect.

Diarrhea involves both an increase in the motility of the gastrointestinal tract, increased gastro intestinal (GIT) secretion and a decrease in the absorption of GIT fluid, the overall effects is reduction in the loss of electrolyte (particularly Na^+) and water¹⁵. Foster and Cox (1983)¹⁶ stated that diarrhea can result from an increase in GFIT motility (Intestinal hurry), which leads to reduction in transit time of fecal materials in the colon. Any substances that decrease intestinal motility (anti-motility) and/or decreases secretion (anti-secretory) will have anti-diarrhea activity.

E. senegalensis and *K. africana* extracts significantly reduced ($P < 0.05$) the motility of isolated rabbit jejunum (Figure 4). They reduced the amplitude of contraction (figure 3 and 4). The observed relaxation of the smooth muscle shows that these extracts possess anti-motility and thus anti-diarrhea activities. The extracts exhibited dose dependent anti-motility effects (Figure 7, 8). It was also observed that graded doses of *E. senegalensis* and *K. africana* significantly ($P < 0.05$) inhibited the enhanced motility effect of acetylcholine (Figure 9, 10).

CONCLUSION

The methanolic extract of *E. senegalensis* and *K. africana* inhibited castor oil induced diarrhea and reduced the spontaneous contraction of the isolated rabbit jejunum. The anti-motility effect of these plants supports the use of these plants in folk medicine for the management of colic pain. The observed effects (antimotility and inhibition of castor oil induced diarrhea) revealed that these extracts may be useful in the management of diarrhea.

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