BEST JOURNAL 4(2):82 - 86
Date received: October, 2007.
Date accepted: October, 2007.
Printed in Nigeria.

ANTIDIARRHEAL ACTIVITY OF THE LEAF EXTRACT OF *STEREOPERMUM KUNTHIANUM*

[*1]U. A. Hanwa, [2]A.H.Kaita, [2]M.I. Sule, [2]A.A. Ahmadu, and [3]M.G. Magaji.

[1]Demonstration Secondary School, Ahmadu Bello University, Zaria.
[2]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria.
[3]Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria.
* Corresponding author: E Mail: Umarhanwa@yahoo.com

ABSTRACT

Preliminary phytochemical screening of the leaves extract of Sterospermum kunthianum revealed the presence of sterols/triterpenes, saponins, tannins, coumarins, and free carboxylic group. The leaves of the plant Stereospermum kunthianum (Bignoniaceae) used in diarrhea treatment in Hanwa Village, Sabon gari, Kaduna State, Nigeria were investigated. The study was carried out on perfused isolated rabbit jejunum and castor oil-induced diarrhea in mice. The ethanolic extract (0.8 3.2mg/ml) causes a dose dependent relaxation of isolated rabbit jejunum. The acute toxicity test for the extract in mice established intraperitoneal LD_{50} of 3807.9mg/kg. In castor oil-induced diarrhea, 60% protection was observed at doses of 500mg/kg and 1000mg/kg respectively. The anti-diarrheal activity was comparable to Loperamide 5mg/kg. The result revealed that the extract have pharmacological activity against diarrhea.

Key words: Stereospermum kunthianum, anti- diarrheal activity, castor oil, tissue relaxation.

INTRODUCTION

Diarrhea is the frequent passage of watery unformed stools. Its causes are many and include irritable bowel syndrome, infectious disorder, thyrotocosis, malabsorption or maldigestion, and laxative abuse. Medications used to treat other disorders also may induce diarrhea. For example. Xanthenes e.g. theophlline preparations cause diarrhea secondary to alteration of mucosal cyclic adenosine monophasphate (cAMP). Antihypertensive drugs, such as reserpine and guanethidine, may induce diarrhea by changing gut neuronal input and reducing noradrenergic mediated relaxation (Craig etal, 1997).

The WHO estimation revealed that diarrhea causes 4 5 million deaths annually throughout the world. 80% of these deaths are reported in developing countries including Nigeria. In Nigeria, diarrheal infection remains the number one killer disease among children under 5 years, while 7 12 month old babies remains the most susceptible (Audu et al, 2002). In addition, reported cases of diarrhea in many areas, including Kaduna state, still account for more than 30% of admissions to children wards (WHO, 1985). Despite the effective and simple cheap treatment of oral dehydration therapy, majority of the local populace still rely on herbs to treat diarrhea (Ahmadu, 2007). The use of herbal drugs in the treatment of diarrhea is a common practice in many developing countries.

In Hausa ethnomedicine of Northern Nigeria, some medicinal plants are used frequently for treating diarrhea infections and these include; *Stereospermum kunthianum*. The plant has been used traditionally in Northern Nigeria as a remedy for diarrhea, dysentery, venereal diseases and as a cure for gonorrhea. (Hutchinson and Dalziel, 1963). It is also a remedy for cough (Brandwijk, 1962). *Stereospermum kunthianum* is used in the treatment of ulcer, leprosy, skin eruptions and pneumonia. The roots and the leaves are used for respiratory ailments and gastritis (Dalziel, 1963). As part of our efforts to screen some ethno medicinal plants of Northern Nigeria for anti diarrheal activity, leaves of *Stereospermum kunthianum* was investigated.

PLANT MATERIALS

Collection and Drying of Plant Materials

Whole fresh plant material bearing fruits and leaves, growing wild was collected at Hanwa village, Sabon-Gari Local Government Area of Kaduna state, Nigeria in the month of December, 2005. The plant was identified by the herbarium unit of the Department of Biological Sciences,

Ahmadu Bello University, Zaria with a specimen Voucher number 1381. The leaves were air dried for 21 days before powdering.

Extraction of Plant Material

The powdered leaf (165g) of the plant was continuously defatted with Petroleum ether (60 80ºC) using Soxhlet extractor until the draining solvent was clear. Solvent used was recovered at reduced pressure to afford a yellowish green waxy material (6.64g) that was thereafter referred to as the petroleum ether extract coded PE. The marc after defatting was air dried at room temperature and then extracted continuously with ethanol using Soxhlet extractor. The Solvent was evaporated at reduce pressure to afford ethanolic extract (10.60g) that was coded EE. This extract was evaluated for its anti diarrheal properties.

Phytochemical analysis

The preliminary phytochemical screening of the ethanolic extract was carried out using the standard procedure (UNIDO, 1970 and Sofowora, 1982).

Animals

New Zealand rabbit weighing 1.5kg and Swiss albino mice 20.0 + 0.5g maintained in the animal house of Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria were used for the experiments. The animals were fed with standard laboratory feeds and water *ad libitum*.

This research was carried out in Ahmadu Bello University, Zaria, Nigeria according to the rules governing the use of laboratory animals as acceptable internationally.

Drugs

Acetylcholine (Sigma chemical, USA), Castor Oil (Bell Sons & Co., England) and Loperamide (Janssen, Germany).

EXPERIMENTAL PROCEDURE

Toxicity Study

The method of Lorke (1983) was adopted. The study was divided into two phases. A total of fifteen mice were used, in phase one, mice were divided into three groups of three mice each with geometrical doses of 10mg/Kg, 100mg/Kg and 1000mg/Kg administered intraperitoneally, the last group received normal saline as a control. No death was recorded after 24 hours. In phase two, 1600mg/Kg, 2900mg/Kg and 5000mg/Kg were administered. The median lethal dose (LD_{50}) was calculated as the geometric mean of the lowest lethal dose and the highest non lethal dose of which there was 1/1 and 0/1 survival.

Effect on Isolated Rabbit Jejunum

The rabbit was sacrificed by a blow on the head, dislocating the neck, exsanguinations. Segments of the jejunum, about 3cm long, were removed and disserted free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode's solution of the following composition in millimoles (mM): NaCl, 136.8; KCl, 2.7; $CaCl_2$, 1.3; $NaHCO_3$, 12.0; $MgCl_2$, 0.5; $NaPO_4$, 0.14; glucose, 5.5. The tissue was mounted in a 25ml organ bath containing Tyrode's solution maintained at 35 1.0 ^{0}C and aerated with air. A lot of 0.5g was applied. A one hour equilibrium period was allowed during which the physiological solution was changed in every 15mins. At the end of the equilibrium period, the effect of acetylcholine (2.0×10^{-8}mg/ml 3.2×10^{-7}mg/ml) an extract of *Stereospermum kumthianum* were investigated non-cumulatively. The contact time for each concentration was one minute, which was followed by washing three times. The tissue was allowed a period of 15mins before the next addition. Responses were recorded isometrically using Ugo Basile recorder 7050 (Amos *et al*., 1998; Agunu *et al*., 2005 and Ahmadu *et al*., 2007).

Effect on 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 5 groups of five mice each. Mice in the first group received 10ml/Kg (ip) normal saline, the second, third and fourth groups received 2000mg/Kg, 1000mg/Kg and 500mg/Kg of ethanolic extract of *Stereospermum kumthianum* (ip), while the fifth group received Loperamide 5mg/Kg (ip). After 30mins of administration of extract, Castor Oil 0.2ml/mouse was administered intragastrically. The animals were placed on individual cages over clean filter paper. Three hours after the administration of oil, the cages were inspected for the presence of characteristic diarrhea droppings. Their absence was recorded as the protection from diarrhea, and the percentage protection calculated (Diurno et al . , 1996; Akah and Offiah, 1996).

Statistical Analysis

The result on Castor Oil-induced diarrhea were analyzed using the Chi Square Test and were regarded as significant when $P<0.05$.

RESULTS

The extraction process yielded 6.42% w/w of ethanolic extract of *Stereospermum kumthianum*. Phytochemical test revealed the presence of sterols/triterpenes, saponins, tannins, coumarins and free carboxylic group (Table 1). The median lethal dose (LD_{50}) of the ethanolic extract in mice (i.p.) was found to be

3807.9mg/Kg. The ethanolic extract of *Stereospermum kumthianum* (2.0×10^{-8}mg/ml 3.2×10^{-7}mg/ml) exhibited a concentration-dependent relaxation of the rabbit jejunum (figure 1). The extract of *Stereospermum kumthianum* (500mg/Kg and 1000mg/Kg) significantly ($p<0.05$) protected the mice against Castor oil-induced diarrhea when compared with the control. This was comparable to that of loperamide (5mg/kg), the standard agents.

Table 1: Phytochemical Constituents of *Stereospermum kumthianum* leaf extract.

Chemical Constituents	Inference
Steroids/terpenes	+
Saponins	+
Tannins	+
Coumarins	+
Flavonoids	-
Alkaloids	-
Free carboxylic group	+

Key: + Present, - Absent

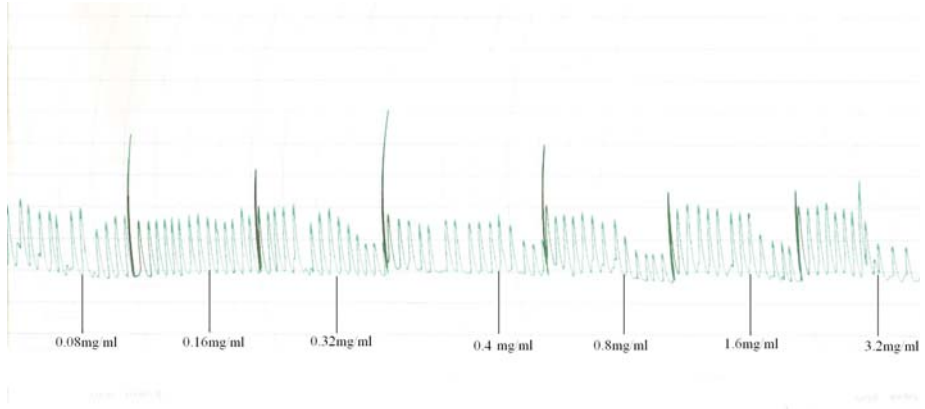

Figure 1: Effect of the ethanolic extract of Stereospermum *kumthianum* on isolated rabbit jejunum

Table 2: The effect of Ethanolic extract of the leaf of *Stereospermum kumthianum* on Castor Oil-induced diarrhea in mice.

Treatment n=5	Dose (*i.p*)	No. of mice with diarrhea	Protection %
Castor Oil	0.2ml/mouse	5/5	0
Stereospermum kumthianum	500mg/Kg	2/5	60
	1000mg/Kg	2/5	60
	2000mg/Kg	5/5	0
Loperamide	5mg/Kg	1/5	80

DISCUSSION AND CONCLUSION

Phytochemical screen of the ethanolic leaf extract of *Stereospermum kumthianum* reveals the presence of steroids/terpenoids, saponins, tannins, coumarins, and free Carboxylic group. The LD_{50} value of the ethanolic extract of the plant was found to be 3807.9mg/Kg (ip) in mice. Castor Oil is made up of 90% ricinoleate (Mekeon et al., 1999) which when metabolized is responsible for the observed effect of the oil. The active metabolites recinoleic acid is responsible for its diarrhea inducing properties, which diminishes Na^+ and Cl^- permeability in the intestine (Gaginella and Phillips, 1975); it is also associated with endogenous stimulation of prostaglandins release (Zavala et al., 1998). Earlier studies showed that anti-dysenteric and anti-diarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, steroids and/or terpenoids are reducing sugars (Anonymous, 1962; Galvez et al., 1991, 1993; Longanga et al., 2000). The anti-diarrheal 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 resistant and hence, reduce secretion (Tripathi, 1994).

Loperamide reduces the daily fecal volume, and decreases intestinal fluid and electrolyte loss. Loperamide produces a rapid and sustained inhibition of the peristaltic reflux through depression of longitudinal and circular muscle activity, presumably through an effect on intestinal opiate receptors. Loperamide is effective against a wide range of secretory stimuli and can be utilized in the control and symptomatic relieve of acute diarrhea that is not secondary to bacterial infection.

In conclusion, the plant extract exhibited anti-diarrheal activity. The effect was comparable to loperamide which is presently one of the most widely used anti-diarrheal drugs and it elicited its activity by antagonizing diarrhea induced by Castor Oil (Niemegeers et al; 1974) and Prostaglandins (Karim and Adaikum, 1997), its therapeutic effect could also be due to its antimotility and its anti-secretory properties (Couper., 1987). The extract similarly inhibited spontaneous agonist induced contractions of rabbit jejunum. The effect may also contribute to the observed anti-diarrheal activity. Tannins have been known to make the intestinal mucosa more resistant and reduce secretion, therefore, inhibit diarrhea induced by Castor Oil (Tripathi, 1994). The presence of tannins in the plant extract could be responsible for the anti-diarrheal activity. The results of this investigation suggest that the leaf extract of *Stereospermum kumthianum* possesses antidiarrheal activity and justify the ethno-medicinal use of the plant in the treatment of diarrhea in Hanwa village, Zaria, Kaduna State Nigeria.

ACKNOWLEDGEMENT

The authors hereby acknowledge the technical assistance of Malam Ibrahim Adamu of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria

REFERENCE

Agunu, A; Sadiq, Y; Gabriel, OA; Zezi, AU and Abdurahman, EM (2005). Evaluation of five Nigerian Medicinal Plants used in treatment of diarrhea in Nigeria. *J. Ethnopharmacol.* 61 : 209 213.

Ahmadu, A. A; Zezi, A.K; and Yaro A. H. (2007). Anti-diarrheal activity of the leaf extracts of Daniellia Oliveri Hutch and Dalz (Fabaceae) and *Ficus sycomorus* Mio (moraceae). *Afr. J. Trad. CAM* 4(4) : 524 528.

Akah, P. A; Offiah, V. N., (1996). Gastrointestinal effects of *Allamanda cathartica* leaf extracts *International Journal of Pharmacognosy*, 30 : 213 217.

Amos, S; Okwusaba, F. K; Gamaniel, K; Akah, P. A. and Wambebe, C. (1998). Inhibitory effects of Gastrointestinal and extracts of *Parefta crassipes* leaves on Gastrointestinal and uterine smooth muscles preparations isolated from Rabbit, Guinea Pigs and Rats. *J. Ethnopharmacol*, 61 : 209 213.

Anonymous, (1962). *The Wealth of India (Raw Material*). CISR. New Delhi, Vol. 6 : 280 281

Audu, R., Umilag, S.A; Renner, J. K; Awodiji (2000). Diarrhea Management. *J. Nigeria Infection Control Association*. 3 : 15

Charles R. Craig and Robert E. Stitzel., (1997). *Modern Pharmacology with Clinical Applications* 5th Edition, Little Brown and Company Boston: 504 506.

Diurno, M. V., Izzo, A. A; Mazzoni, B; Bologgnese, A., Capasso, F; (1996). Anti-diarrheal activity of New thiazolidinones related to Loperamide. *Journal of Pharmacy and Pharmacology* 48; 760 762

Galvez, J. Zarzuelo A. Crespo, M. E. (1991). Anti-diarrheal activity of *Silerocarya birreh* bark extract and its active tannin constituent in rats*. Phytother Res*. 5 : 276 278.

Galvez, J. Zarzuelo A. Crespo, M. E; Lorente, M.D; Ocets, M. A.; Jimenez J; (1993). Anti-diarrheal activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. *Planta Medica,* 59 : 333 336.

J. Hutchinson and J. M. Dalziel, (1963). *Flora of West Tropical Africa*, Vol. II : The Whitefrians Press Limited London. Pp 21-26

Longanga Otshudi, A; Vercruysse, A; Forrier A (2002). Contribution to ethno botanical, Phytochemical and Pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in Lomda area of the Democratic Republic of Congo (DRC). *J. Ethnopharmacol*. 71(3) : 411 423.

Lork, D. (1983). A new approach to acute toxicity testing. Arch Toxicol. 54 : 275 287

Mekeon, T. A; Lin, J. J; Stafford, A.E; (1999). Biosynthesis of ricinoleate in Castor Oil. *Adv.Exp.Med. Biol*. 46437 46447

Sofowora, A; (1982). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Limited Ibadan.Pp.34-42

Tripathi K. D. (1994). *Essentials of Medical Pharmacology*. Jay Pee Brothers Medicals Publishers (P), New Delhi P. 775

UNIDO (1970) *Methodology for Analysis of Vegetable Drugs,* Chemical Industrial Branch Division of Industrial operation

WHO, 1985. *The Management of Diarrhea and the use of Oral Dehydration Therapy*: A joint WHO/UNICEF Statement second edition Geneva

Zavala, M. A; Perez, C., Vargal, R; Perez, R. M. (1998). Anti-diarrheal activity of *Waltheria americana*, *Commelins cuelestris* and *Alternanthra repens*; *J. Ethnopharmacol*., 61 : 41 - 47