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ANTI-DIARRHEAL ACTIVITY OF THE LEAF EXTRACTS OF *DANIELLIA OLIVERI* HUTCH AND DALZ (FABACEAE) AND FICUS SYCOMORUS MIQ (MORACEAE)

A. A. Ahmadu[a]*, A. U. Zezi[b], and A. H. Yaro[b].

[a] Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria-Nigeria., [b]Department of pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria
E-mail: ahmadu2001@yahoo.com

Abstract

The leaves of the plants *Daniellia oliveri* (Fabaceae) and *Ficus sycomorus*(Moraceae) used in diarrhea treatment in Hausa ethnomedicine of Northern Nigeria were investigated. The study was carried out on parfused isolated rabbit jejunum and castor oil-induced diarrhea in mice. The n-butanol extracts: NBD and NBF (0.16-3.2mg/ml) caused a dose-dependent relaxation of isolated rabbit jejunum. The acute toxicity test for NBD and NBT in mice established an i.p LD_{50} of > 4000mg/kg for *D. oliveri* and 1131.4mg/kg for *F. sycomorus.* In castor oil-induced diarrhea, 80% protection was observed for *D. oliveri* at doses of 200mg/kg and 60% protection was observed at 100mg/kg and 50mg/kg respectively. For *F. sycomorus* 100% protection was observed at doses of 120mg/kg and 60mg/kg, for the n-butanol extract. The antidiarrheal activity was comparable to loperamide 5mg/kg. The result revealed that the extracts have pharmacological activity against diarrhea.

Key words: Anti-diarrhea,castor oil,n-butanol extracts,tissue relaxation.

Introduction

Diarrhea is still one of the major health threats to population in tropic and subtropical countries (Heinrich, 2005). In Nigeria, it remains the number one killer diseases among children under 5 years, while babies between the ages of 7 – 12 months remain the most susceptible (Audu et al., 2000). The WHO has estimates that 3 - 5 billion cases occur each year with 1 billion in children below the age of 5 and 5 million deaths result from diarrhea annually with 50% in children below the age of five (Abdullahi et al., 2000). Despite the effective and simple cheap treatment of oral dehydration therapy, majority of the local populace still rely on herbs to treat diarrhea.

In Hausa ethnomedicine of Northern Nigeria, some medicinal plants are used frequently for treating diarrhea infections and these include: *D. oliveri* Hutch and Dalz (Fabaceae) and *F. sycomorus* Linn (Moraceae). The leaves of *D. oliveri* are used traditionally in Northern Nigeria to treat diabetes, gastrointestinal disturbances, diarrhea, as diuretic and aphrodisiac (Hutchinson and Dalziel, 1964; Onwukaema and Udoh, 1998), while the leaves of *F. sycomorus* are used in Hausa ethnomedicine of northern Nigeria to treat dysentery, diarrhea, cough and chest conditions (Sandabe and Kwari,2000; Hutchinson and Dalziel,1964)). As part of our efforts to screen some ethnomedicinal plants of Northern Nigeria for antidiarrheal activity the leaves of *D. oliveri* and *F. sycomorus* were investigated.

Materials and Methods

Collection and Preparation of plant materials

The leaves of the two plants were collected in Samaru, Zaria-Nigeria in August, 2004. The plants were identified by Taxonomical means and authenticated by U. Gallah at the herbarium unit of the Department of

Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, and voucher specimens: (6907) for *D. oliveri* and (6908) for *F. sycomorus* were deposited at the herbarium. The air-dried powdered plants (300g each) were extracted with 70% ethanol at room temperature. The two extracts were concentrated to obtain the dried extract (Brain and Turner, 1975; Sofowora, 1993). The aqueous ethanolic extract was suspended in water and partitioned with ethylacetate and n-butanol to give 0.7g and 5.2g for *D. oliveri* and 1.2 and 2.4g for *F. sycomorus.*

Phytochemical analysis

The Extracts were subjected to phytochemical analysis for constituent identification using standard protocol (Silva et al, 1998).

Animals

New Zealand rabbit weighing 1.6kg and Swiss albino mice 20.0 $\pm$ 0.5g maintained in the animal house of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria-Nigeria were used for the experiments. The animals were housed in steel cages under standard conditions and fed with standard laboratory feedsand water *ad libitum.* All procedures were carried out in accordance with the institutional animal care and use.

Experimental Procedure
Toxicity studies

The method of (Lorke, 1983) was adopted. A batch of 15 mice in each study was used. In phase I, mice were divided into four groups of three mice each with geometric doses of 10mg/kg, 100mg/kg and 1000mg/kg administered intraperitoneally, the last group received normal saline as the control. No death was recorded after 48 hours. In phase II, 200mg/kg, 400mg/kg, 800mg/kg and 1600mg/kg was administered.

Effect on Extracts on Isolated Rabbit Jejunum

The rabbit was sacrificed by a blow on the head, segment of the jejunum (2 – 3 cm long) were removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode solution of composition: (mM) NaCl 136.8, KCl 2.7, $CaCl_2$ 1.3, $NaHCO_{3\ 12.0,}$ $MgCl_2$ 0.5, $NaPO_4$ 0.14 and glucose 5.5. The tissue was mounted in a 25ml organ bath containing tyrode solution maintained at 35 ± 1.0^0C and aerated with air. A load of 0.5g was applied. A 1 hr equilibrium period was allowed during which the physiological solution was changed every 15 mins. Effects of acetylcholine (2.0×10^{-8}g/ml – 3.2×10^{-7}g/ml) and extracts of *D. oliveri* and *F. sycomorus* were investigated non-cumulatively. The contact time for each concentration was 1 min, which was followed by washing the tissue three times. The tissue was allowed to rest for 15 mins before the next addition. Responses were recorded isometrically using Ugo Basile recorder 7050 (Amos et al., 1998; Agunu et al., 2005).

Effects on Castor oil-induced diarrhea in Mice

The mice were fasted 12 hours prior to the commencement of the experiments and were randomly divided into five groups of five mice each. Mice in the first group received 10ml/kg (i.p) normal saline, the second, third and the fourth group received 200mg/kg, 100mg/kg and 50mg/kg of n-butanol extract of *D. oliveri* (i.p), while the fifth group received loperamide 5mg/kg (i.p). After 30 mins 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 (by an observer unaware of the particular treatment) for the presence of characteristic diarrhea droppings. Their absence was recorded as a protection from diarrhea, and the percentage protection calculated (Diurno et al., 1996; Akah and Offiah, 1996). The same procedure was repeated for N-butanol extract of *F. sycomorus* at doses of 120mg/kg, 60mg/kg and 30mg/kg base on the LD_{50}.

Statistical Analysis

The results on castor oil-induced diarrhea were analysed using Chi-Square test and were regarded as significant when $P < 0.05$.

Results

The extraction process yielded 8.3%w/w and 10.3%w/w of ethanolic extract of *Daniellia oliveri* and *Ficus sycomorus* respectively. Phytochemical tests showed that both extract tested positive to carbohydrates, reducing sugars, flavonoids, steroids/terpenoids (Table 1).The acute toxicity studies for the N-butanol extract in mice (i.p) was found to be 1141.4mg/kg and > 4000mg/kg for *F. sycomorus* and *D. oliveri* respectively. These two extracts were used for the pharmacological investigations.

Effect of extract on rabbit jejunum

The effect of n-butanol extracts of *D. oliveri* (0.4 – 3.2mg/ml) on the rabbit jejunum were dose-dependent (Figure 1);similarly that of *F. sycomorus*(0.16mg/ml-2.56mg/ml) was also dose-dependent (Figure 2).

Effect of extract on castor oil induced diarrhea

The N-butanol extract of both plants inhibited castor oil-induced diarrhea in mice, however mice pre-treated with *F. sycomorus* extract showed better protection than that of *D. oliveri* (Table 2).

Table 1: Phytochemical constituents of *Daniellia oliveri* and *Ficus sycomorus* Leaves

Chemical constituents	*Daniellia Oliveri*	*Ficus sycomorus*
Steroids/terpenes	+	+
Alkaloid	-	-
Carbohydrates/ Sugars	+	+
Flavonoids	+	-
Anthraquinones	-	-
Tanins	+	+

Key:
+ Present
- Absent

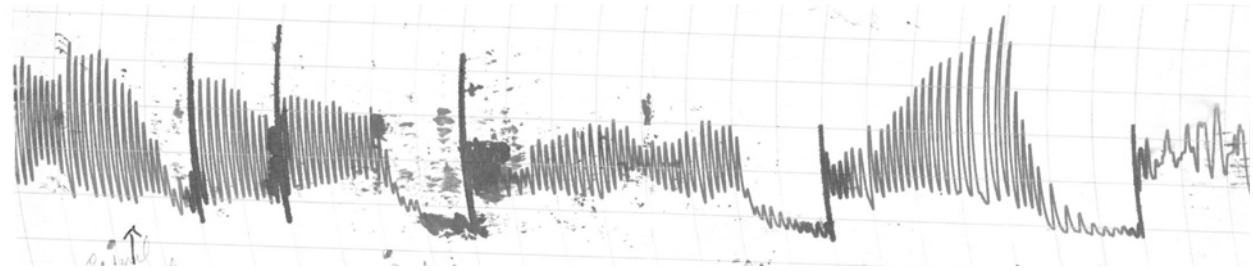

Figure 1: The effect of N-butanol extract of the leaves of *D. oliveri* on the isolated ileum of the rabbit

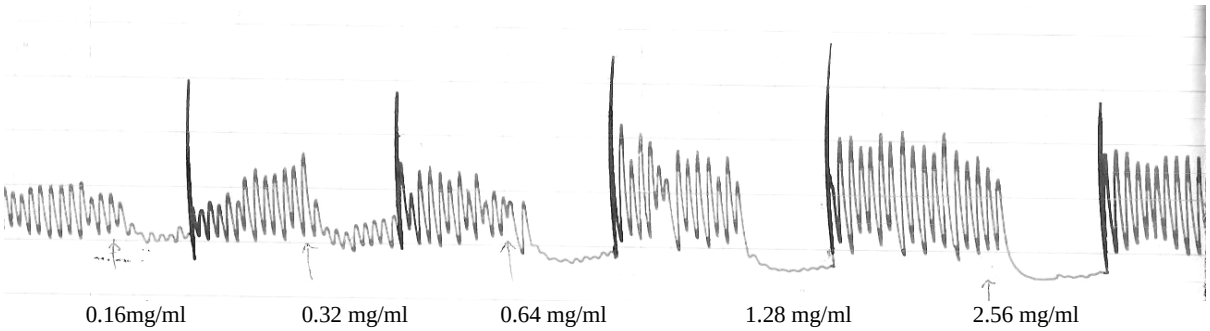

Figure 2: The effect of N-butanol extract of the leaves of *F. sycomorus* on the isolated ileum of the rabbit

Table 2: The effect of n-butanol extracts of *Daniella oliveri* and *Ficus sycomorus* on Castor oil – induced diarrhea in mice

Treatment	Dose (i.p)	Number of mice with diarrhea	Protection (%)
Castor oil	0.2ml/mouse intragastrically	5/5	0
Daniellia oliveri[a]	50mg/kg	2/5	
	100mg/kg	2/5	40
	200mg/kg	3/5	60
			80
Ficus sycomorus[a]	30mg/kg		
	60mg/kg	2/5	
	120mg/kg	0/5	60
		0/5	100
			100
Loperamide	5mg/kg		
		1/5	
			80

Effect of Castor oil – induced diarrhea

The extracts of *D. oliveri* (50, 100 and 200mg/kg) and loperamide gave significant protection ($P < 0.05$) on mice against castor oil–induced diarrhea when compared with the control. Highest protection was observed at 200mg/kg. Similarly, the extract of *F. sycomorus* also showed significant protection ($P < 0.05$) in mice against castor oil-induced diarrhea at doses of 30mg/kg, 60mg/kg and 120mg/kg when compared with control. At 60mg/kg the ethylacetate extract gave 80% protection, while n-butanol extract retained the maximum protection.

Discussion and Conclusion

The two plant extracts exhibited anti-diarrheal activity, however, *F. sycomorus* showed better activity at 120mg/kg and 60mg/kg for n-butanol extracts and 60mg/kg. These effects were 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 Adaikun, 1997), its therapeutic effect could also be due to its antimotility and antisecretary properties (Couper., 1987). The extracts similarly inhibited spontaneous agonist induced contractions of rabbit jejunum. These effects may also contribute to the observed anti-

diarrheal activity. Flavonoids have been known to inhibit contractions induced by spasmogenes (Macauder, 1986; Matd et al., 1997). Similarly, they have been known to inhibit diarrhea induced by castor oil (Galvez et al., 1993). Flavonoids have also been reported to have antimicrobial activities (Bylka et al., 2004). The presence of flavonoids in both plant extract could be responsible for their anti-diarrheal activity. This justifies the ethnomedicinal use of the plants.

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