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Bioassay-guided evaluation of the antidiarrhoeal potentials of *Zizyphus spina-christi* rootbark in rats

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ABSTRACT

The basis for the use of *Zizyphus spina-christi* in folkloric practice as a remedy for diarrhoea was investigated in rats. The rootbark of the plant material was sequentially extracted and the main antidiarrhoeal activity was traced to a fraction (numbered ZS-4E), eluted with chloroform-methanol (60:40), from the methanol extract. The fraction was tested for antidiarrhoeal activity against castor oil induced-diarrhoea and fluid accumulation, and charcoal meal test in rats. Results show that the fraction (25, 50 and 100 mg/kg, p.o.) exhibited some level of efficacy against the models used which however was not dose-dependent. Phytochemical tests of the methanol extract indicated the presence of tannins, saponins, balsams and carbohydrates. The total tannin content was established to be 18.1 mg/g.

Keywords: Fraction ZS-4E, castor oil-induced diarrhea, fluid accumulation, gastrointestinal transit test.

INTRODUCTION

In line with its widespread use and popularity, the World Health Organisation (WHO) has recommended the integration of traditional medicines proven to be useful into national health care programmes (WHO, 1976). The organisation has also set up guidelines for its study (WHO, 1991) and defined its role under what it termed 'Traditional Medicine/Complementary and Alternative Medicine (TM/CAM)' by developing a strategy to address issues of policy, safety, efficacy, quality, access and rational use (WHO Traditional Medicine Strategy 2002-2005). The practice, mainly involves the use of medicinal plants as complex mixtures containing a broad range of constituents or as pure chemically defined active principles (Hamburger and Hostettman, 1991). In view of this widespread usage, important technical aspects such as standardization and quality control need development (Farnsworth, 1980; De Smet, 1991).

Zizyphus spina-christi Willd (Rhamnaceae) has been used in traditional medical system as a remedy for diarrhoea (Al-Yahya, 1986; Adzu et al., 2003). We have earlier reported the activity of the methanol extract of the crude stem bark (Adzu et al., 2003). In reporting that of the rootbark in this study, we first sequentially extracted the plant

material with four solvents along increasing polarity with hexane, chloroform, ethylacetate and methanol using a soxhlet extractor (Quickfit, England). The extracts were preliminary tested via monitoring of antidiarrhoeal activity. Ethylacetate and methanol extracts gave the most potent effect. In view of the low yield (4.43 g) of the ethylacetate extract (equivalent to 0.34 %w/w of crude starting material), we decided to concentrate on the methanol extract (yield 18.99%). The methanol extract was fractionated using flash column chromatography, and the main antidiarrhoeal activity was traced to the fraction eluted with chloroform-methanol (60:40). The fraction was then fully investigated against castor oil-induced diarrhoea, castor oil-induced fluid accumulation, and small intestine transit test in rats. The phytochemical constituent of the methanol extract was also tested as well as its total tannin content.

MATERIALS AND METHODS

Plant material and extraction

The plant materials were collected locally at Midlu, Adamawa State, Nigeria from April to May, 2004, and were authenticated at Taxonomy Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (NIPRD #4108) was kept at the herbarium of the Institute. The root bark of the plant was removed, cleaned and dried under shade. The dried root was ground into powder and 1.29 kg of the powdered material was sequentially extracted to obtain hexane (ZS-1), chloroform (ZS-2), ethylacetate (ZS-3) and methanol (ZS-4) extracts. The combined extract of each solvent was concentrated over a water bath and evaporated to dryness at room temperature. The root bark gave yields of 4.623 g (0.33%), 27.834g (2%), 4.443 g (0.32%) and 245 g (17.63%) for hexane, chloroform, ethylacetate and methanol extracts respectively. The total yield of the extracts was 21.86% w/w of crude starting material.

Phytochemical test

Phytochemical constituents present were screened using standard procedures (Harborne, 1998; Evans, 2002). Total tannin content of the methanol extract was quantified using the Lowenthal Permanganate Titration method (Burroughs and Whitting, 1960) as earlier adopted (Adzu et al., 2004).TLC was carried out to determine spot zones which were viewed under UV light (254/365nm Eagle Scientific Ltd, UK).

Fractionation of extracts

The methanol extract (20 g) was fractionated with flash column (Still et al., 1978) using silica gel (Aldrich Chemicals; 230-400 Mesh). It was eluted with solvents of increasing polarities, starting with hexane, combinations of chloroform and methanol, and methanol in multiples of 100 ml using a pump (ABM, Germany). Fractions of 100 ml were collected separately. Fractions having the same TLC profile were combined together, concentrated over a water bath, and allowed to evaporate at room temperature. This resulted in 6 fractions (ZS-4A to ZS-4F): ZS-4A (0.617 g); ZS-4B (0.047 g); ZS-4C (0.135 g); ZS-4D (0.921 g); ZS-4E (0.324 g) and ZS-4F (13.97) totaling 19.01 g (95% recovery).

Chemicals, drugs and test agents

Hexane, chloroform, methanol (Sigma-Aldrich, Germany), ethylacetate (BDH Chemicals Ltd, England), Lomotil® (Searle, England), tragacanth powder (Bush, Boake Allen, England) and castor oil (Sigma Chemical Co., USA) were used. Three dose levels of the fraction (25, 50 and 100 mg/kg) were used and administered orally (Aikawa, 2000). Diphenoxylate (2.5 mg/kg, p.o.) was used as the positive control, while saline (for castor oil-induced diarrhea) or 10% aqueous tragacanth (10 ml/kg, p.o.) (for charcoal meal test) was used as the negative control in all the experiments.

Animals

Six to eight weeks old male wistar rats (Aikawa, 1999), weighing 180 – 230g obtained from the Animal Facility Centre (AFC), NIPRD, Abuja were used according to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 83-27, 1985) in accordance with the principles of Good Laboratory Procedure (GLP) (WHO, 1998) as contained in NIPRD's Standard

Operational Procedures (SOPs). The rats were fasted for 18 h prior to all the experiments.

Antidiarrhoeal studies

Castor oil-induced diarrhoea and decrease in body weight

A total of 30 rats were used in this test, according to the procedure described by Awouters et al. (1978), modified for our local laboratory settings (Adzu et al., 2003; 2004). The rats were weighed, divided into five groups (n = 6) and treated with ZS-4E, saline or diphenoxylate. Each rat was given 1 ml (p.o.) of castor oil (Mabeku et al., 2006), and 30 min later was placed in an observation cage lined with sheets of paper. The diarrhoea score of each rat was taken 3 h after the castor oil administration and recorded using a predetermined scoring index (Di Carlo et al., 1994) as follows; (+ +) for copious, (+) for mild and 0 for lack of diarrhoea. The animals were reweighed once again, and changes in body weigh taken (Yu et al., 2000). The total score of each group were taken and activity expressed as % inhibition of diarrhoea.

Castor oil induced intestinal fluid accumulation

This test (Robert et al., 1976; Di Carlo et al., 1994) was similar to the one described above (2.6.1.) except that in this procedure, each rat received 2 ml (p.o.) of castor oil after treatment and were killed 30 min later by inhalation of chloroform. After which the abdomen was opened, small intestine carefully removed, dissected, its content from pylorus to caecum expelled into a petridish, drawn into a syringe and measured (Adzu et al., 2004).

Small intestine transit test

The procedure adopted for this test was similar to the one described by Capasso et al. (1976). Rats used were grouped and treated with extract, diphenoxylate or aqueous tragacanth. They were each given 0.5 ml of 5% deactivated charcoal suspended in 10% aqueous tragacanth powder (p.o.), 30 min after treatment. The rats were then killed 30 min later by inhalation of chloroform, the abdomen opened, small intestine removed and the distance traveled by charcoal plug from the pylorus measured (Abdullahi et al., 2001).

Statistical analysis of data

All results were expressed as mean ± SEM. Student test was used to analyse the result between groups; $p < 0.05$ was taking as level of significance in all cases. Chi-square was used to analyse the diarrhoea score.

RESULTS

Standard test for the phytochemical constituents of the methanol extract of *Zizyphus spina-christi* revealed the presence of tannins, saponins, balsams and carbohydrates. Tannin content was established to be 18.1 mg/g.

The fraction (ZS-4E) exhibited some protection against the castor oil induced diarrhoea for as much as 66.67% in the group that received 50 mg/kg, p.o. of the fraction. The effect was however not dose-dependent. The entire group showed some decrease in their body weight at the end of the experimental time (Table 1). The fraction also inhibited the intestinal fluid accumulation (Table 2) and showed some inhibitory effect on the charcoal meal motility test showing an inhibition of 27–34%. This effect was also not dose-dependent (Table 3).

Table 1: Effect of fraction ZS-4E and diphenoxylate against castor oil induced diarrhea

Treatment	Dose (mg/kg, p.o.)	Loss in b.w. (g)	Diarrhoea score				Inhibition[a] (%)
			++	+	0	Total	
Saline (10 ml/kg, p.o)	-	4.83 ± 1.38	6	0	0	12	-
ZS-4E	25	4.80 ± 3.7	3	3	0	9	25
	50	4.53 ± 1.63	1	2	3	4	66.67
	100	3.95 ± 0.70	2	1	3	5	58.33
Diphenoxylate	2.5	2.75 ± 1.75	0	2	4	2	88.33

b.w. = body weight; [a] Compared with saline control

Table: 2: Effect of fraction ZS-4E and diphenoxylate against castor oil induced intestinal fluid accumulation in rats

Treatment	Dose (mg/kg, p.o)	Fluid volume (ml)	Inhibition (%)
Saline (10 ml/kg)	-	1.88 ± 0.23	-
ZS-4E	25	1.31 ± 0.29*	30.32
	50	0. 67 ± 0.17*	64.36
	100	0. 62 ± 0.22*	67.02
Diphenoxylate	2.5	0. 48 ± 0.20*	74.47

* denote significant differences between treated and control groups. $p < 0.05$

Table 3: Effect of fraction ZS-4E and diphenoxylate on transit (charcoal meal) motility test in rats

Treatment	Dose (mg/kg, p.o.)	Distance traveled by charcoal plug (cm)	% Inhibition
Saline (10 ml/kg, p.o.)	-	57 ± 3.85	-
ZS-4E	25	41.75 ± 6.32	26.75
	50	37.88 ± 4.06	33.54
	100	39.75 ± 4.24	30.26
Diphenoxylate	2.5	18.38 ± 1.3	67.75

DISCUSSION

Diarrhoea, especially those of infectious origin is a major health problem in developing countries. The infections mainly caused by enteroxigenic bacteria and often predisposed by malnutrition is still a threat to public health (Cos et al., 2006). The diarrhea-causing substances do that by increasing the osmotic influx of water and ion to the intestinal lumen (Velazquez et al., 2006). A lot of plant constituents have been noted to exhibit antidiarrhoeal effect (Rios and Recio, 2005) and the WHO has even recognized that (Syder and Merson, 1982).

Diarrhoea can be experimentally induced using castor oil. The diarrhoea inducing property of the oil is believed to be due to its active component ricinoleic acid (Mckeon et al., 1999) which causes irritation that diminishes electrolyte permeability in the small intestine, associated with endogeneous stimulation of prostaglandins release (Ganigella and Phillips, 1975; Zavala et al., 1998). It is as the result of the production of several mediating substances that include prostaglandins, nitric oxide, and platelet activating factor, cAMP and tacykinins (Izzo et al., 1998). ZS-4E inhibited the castor oil-induced diarrhoea and fluid accumulation. Agents that exhibit such activity have antidiarrhoeal effect, and do that through antielectrolyte permeability effect and/or inhibition of gastrointestinal tract functions (Nwafor et al., 2002). The fraction also exhibited an ability to inhibit small intestine propulsion in the charcoal meal motility test. Again, such effects are attributed to antidiarrhoeal activity (Di Carlo et al., 1994; Mabeku et al., 2006), plausibly by inhibition of the gastrointestinal tract function (Nwafor et al., 2002) as collaborated by the observed decrease in body weight of the rats (Yu et al., 2000).The activity of the extract didn't seems to be dose dependent, but tend to act similar to the reference drug (diphenoxylate) used. Diphenoxylate, an opioid antidiarrhoeal act by reducing the transmission of nerve signals to the intestinal muscles, thus reducing muscles contraction allowing more time for water to be absorbed from food residue thereby reducing fluidity as well as the frequency of bowel movement (Peters, 2004).

Overall, the extract exhibited antidiarrhoeal activity which may be due to an inhibitory action against gastrointestinal motility and/or an antienteropooling effects (Shook et al., 1989). The former may be due to the reasons postulated above, while the latter may probably be as a result of its tannin contents. Tannins form protein tannates,

which reduce secretion and make the intestinal mucosa more resistant (Tripathi, 1994). The earlier reported antibacterial activity of the plant's root bark (Adamu et al., 2005) might also contribute to its claimed efficacy, and usefulness during infectious diarrhoea. Conclusively, these results established the fact that the root bark of *Zizyphus spina-christi* has antidiarrhoea activity. This activity was in this study traced to a fraction eluted with chloroform-methanol (60:40) from the methanol extract.

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