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Evaluation of *Anacardium occidentale* Methanol Leaf Extracts in Experimental Diarrhoea of Mice

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SUMMARY

Oral administration of various doses (100, 200 and 400 mg/kg) of methanol extracts of the leaves of Anacardium occidentale produced significant antidiarrhoeal activities in mice by reducing the number and frequency of defecation of wet faeces, reduction in intestinal weight and intraluminal fluid volume as well as reducing the intestinal transit in charcoal meal test when compared to diphenoxylate Hcl (5mg/kg, p.o.) control in a dose dependent manner. The highest dose (400 mg/kg) produced better antidiarrhoeal activity than the reference drug, diphenoxylate. Findings from the study reveal that Anacardium occidentale leaf extracts possess antidiarrhoeal principles validating its traditional use in the management of diarrhoea.

KEY WORDS: Anacardium occidentale, Antidiarrhoeal activity, Intestinal transit, castor oil induced diarrhoea, castor oil-induced enteropooling.

INTRODUCTION

In developing countries, there are large numbers of epidemiological and experimental indications pertaining to acute-diarrhoeal disease, a foremost cause of infant death majorly associated with malnutrition (instead of -----by associated malnutrition). In an attempt to reduce (wipe out) the problem of diarrhoea, which is a major cause of increasing mortality rate in developing countries, the World Health Organisation has formed a Diarrhoea Disease Control Program (CDD) which includes traditional remedies, health education evaluation as well as prevention approaches (Biswas et al., 2002). It thus becomes important to identify and evaluate commonly available natural drugs as alternative to currently used antidiarrhoeal drugs, which are not completely free from adverse effects (Etuk et al., 2009). A range of medicinal plants with anti-diarrhoeal properties has been widely used by the traditional healers; studies of some have shown the effectiveness of some traditional medicines in treating diarrhoea (Agunu et al., 2005). However, most traditional remedies have not been scientifically evaluated.

Anacardium occidentale (Anacardiaceae), commonly known as Cashew, is a widely grown plant in South eastern Nigeria has been reported to possess a number of medicinal properties and other purposes (Sokeng et al., 2001; Tedong et al., 2006; Ramesh-Kannan et

al., 2009). On the basis of traditional use of the plant as a potent anti-diarrhoeal agent, this study was carried out with methanol extracts of the leaves of A. occidentale to validate the claims of traditional healers using castor oil-induced diarrhoea model.

MATERIALS AND METHODS

Plant Material

The leaves of Anacardium occidentale were collected around Isieke Afaranta, Ibeku-Umuahia, Abia State, Nigeria in November, 2010. They were taxonomically identified by Dr. M.C. Dike of the forestry department of the Michael Okpara University of Agriculture, Umudike. Voucher specimen (VPP/CVM/MOUAU/30/2010) has been deposited at the herbarium of the Department of Veterinary Physiology, Biochemistry and Pharmacology, Michael Okpara University of Agriculture, Umudike.

Preparation of the Extract

The leaves were dried under shade and comminuted into coarse powder and 100 g of the dried leaves were extracted with 80% MeOH and concentrated in vacuo (yield: 7.13% on dried wt.). Preliminary qualitative phytochemical analysis of A. occidentale (AO) show the abundant presence of carbohydrates, volatile oils, phenols, saponins, tannins, terpenes and flavonoids in trace amounts.

Animals

Albino Swiss mice of either sex weighing 23 - 45 g were used for castor oil-induced anti-diarrhoeal, anti-secretory and intestinal transit activity. All animals were fed standard animal feed (Vital feed®, Nigeria) and clean drinking water ad libitum before the experiments. Each experimental group consisted of six animals housed in separate cages.

Acute Toxicity Test

The method of Lorke (1983) was employed in this test. Twenty five mice of both sexes were randomly grouped into five of five mice each and were fed orally with graded doses (100, 500, 1000, 1500 and 2000 mg/kg of the extract by gastric gavage. The animals were allowed free access to fed and water. They were observed over a 48 h period for acutely toxic signs and death.

Castor Oil-Induced Diarrhoea

The methods described by Ezekwesili et al. (2004) were employed. Mice were divided into five groups of six animals each, diarrhoea was induced by administering 1 ml of castor oil orally to mice. Group 1 served as control (10 ml/kg, p.o. distilled water), group 2 received diphenoxylate Hcl (5 mg/kg, p.o.) served as standard and group 3, 4, and 5 received extract (100, 200 and 400 mg/kg, p.o.) 1 h before castor oil administration. The number of both wet and dry diarrhoeal droppings were counted every hour for a period of 4 h. Mean of the wet stools passed by the treated groups were compared with those of the control groups.

Castor Oil-Induced Enteropooling

Intraluminal fluid accumulation was determined by the methods of Robert et al. (1976). Animals were fasted overnight and divided into five groups of six animals each. Group 1 received distilled water (10 ml/kg, p.o), served as a negative control, group 2 received atropine (3mg/kg, i.p., Positive control) and groups 3, 4 and 5 received the extract of 100, 200 and 400 mg/kg orally respectively 1h before the oral administration of castor oil. Two hours later the animals were sacrificed by cervical dislocation, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volumes were measured. The intestine was reweighed and the difference between full and empty intestines was calculated.

Effect on Small Intestinal Transit

Mice were fasted for 18 h divided into five groups of six animals each, Group1 received 2

ml of castor oil orally with distilled water 10 ml/kg orally. Group 2 received diphenoxylate Hcl (5mg/kg, p.o.) groups 3, 4 and 5 received 100, 200 and 400 mg/kg orally of the plant extract respectively, 1 h before administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The animals were sacrificed after 1h and the distance travelled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum (Mascola et al., 1994; Chidume et al., 2001).

Statistical Analysis

Data obtained were represented as mean ± S.E.M. (Standard error of the mean). ANOVA and post hoc LSD were used for frequency of defecation, the wetness of the evaluation of data and $P<0.05$ were considered significant at 95% confidence limit (Woodson, 1987).

RESULTS AND DISCUSSION

No deaths were recorded after 48 hrs of administration of the various doses (100, 500, 1000, 1500 and 2000 mg/kg) of the methanol leaf extract of Anacardium occidentale. All the rats treated with the extract were dull; clustered together for about seven hours after the oral administration of the extract. The rats recovered 10 hours post administration and became very active for the remaining 38 hours.

The anti-diarrhoeal properties of Anacardium occidentale was studied using models justified by works of Havagiray, et al., (2004) such as castor oil induced diarrhoea, charcoal meal transit time and castor oil induced enteropooling in mice. In some diarrhoea, the secretory constituent predominates while other diarrhoeas are considered on the basis of gastrointestinal tract hyper-motility.

Previous studies show that activated charcoal readily adsorbs drugs and chemicals on intestinal surfaces, thereby preventing absorption (Levy, 1982), justifying the use of charcoal meal to study the effect of leaf extract of A. occidentale on peristaltic movement. Oral administration of 100, 200 and 400 mg/kg doses of the methanol extract of A. occidentale leaves to mice and the reference drug diphenoxylate significantly ($P < 0.05$) reduced the frequency of defecation, the wetness of faecal droppings (Table 1). The distance travelled by the charcoal plug through the small dependent manner, and the highest dose of the extract (400 mg/kg) significantly decreased charcoal meal thrust from 71 to 34% when compared with the negative control ($P <0.05$) (Table 2). According to Bruton (1996), the reduction of intestinal contractions and associated intestinal transit is observed with most antidiarrhoeal drugs, as with the extract. Reduction of intestinal transit time may possibly be due to anti-cholinergic effects, if any. Diphenoxylate

TABLE I: Effect of the extract of *Anacardium occidentale* on castor oil-induced diarrhoea

Groups	Treatment	Faeces (n/mice)	Wet Faeces (n/mice)	% Antidiarrhoeal Activity
1	Dist. Water(10ml/kg)	4.02± 0.01	15.03± 0.12	78.95
2	Diphenoxylate (5mg/ kg)	21.60±0.65	5.12± 0.11	19.23
3	AO (100 mg/kg)	13.22± 0.20*	5.03± 0.01	26.87
4	AO (200 mg/kg)	12.02± 0.10*	5.07± 0.21	19.47
5	AO (400 mg/kg)	18.01± 0.03	6.12± 0.08*	15.08

AO means *Anacardium occidentale* leaf extract. Values are expressed as mean ±SEM; * show that values are significantly different from positive control, Diphenoxylate ($p <0.05$)

used in the in the symptomatic control of diarrhoea, works through its anti-motility effects (Aliu, 2007). A. occidentale extract may also be acting via the same mechanism.

Castor oil caused accumulation of water and electrolytes in intestinal loop by prevention of the re- absorption of water. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa leading to release of prostaglandins which results in stimulation of motility and secretion and the prevention of re-absorption of NaCl and water (Pierce *et al.*, 1971). The reference drug atropine (3 mg/kg, i.p.) and all doses of the extract produced a dose-dependent reduction in intestinal weight and intraluminal fluid volume. 100 mg/kg, p.o. dose of extract produced 44.17% inhibition of volume of intestinal content (P<0.01), while, 200 and 400 mg/kg, p.o. dose produced 48.47 and 66.24% inhibition of volume of intestinal content respectively (P<0.05). The weight of intestinal content was also reduced significantly (P<0.01) at all the doses employed (Table 3).

The effect of *Anacardium occidentale* on castor oil induced enteropooling showed that the reference drug atropine (3 mg/kg) and the extract in all the doses used significantly ($P < 0.05$) reduced the intraluminal fluid accumulation in a dose dependent manner. The prevention of intraluminal fluid secretion by *A. occidentale* in this study may be due to inhibition of prostaglandin biosynthesis with resultant decrease in secretion of fluid into the lumen or may be due to promotion of absorption of water and electrolytes in the gut. Suppression of intestinal fluid accumulation by the extract might also suggest inhibition of gastrointestinal function (Nwafor *et al.*, 2000).

Phytochemical screening of the aqueous extract of *A. occidentale* revealed the presence of phenols, glycosides, flavonoids, steroids and triterpenes, while alkaloids, saponins and tannins were absent (Fazali *et al.*, 2011). Some of the chemical constituents present in the leaf extract have been shown to have antidiarrhoeal activity.

Previous studies have incriminated tannins, flavonoids, reducing sugars/glycosides among others as potent antidiarrhoeal and antidysentery agents (Loganga *et al.*, 2000; Palombo, 2005). Flavonoids have also been

TABLE II: Effect of *A. occidentale* extract on charcoal transit time in mice (mean ±SEM)

Group	Treatment	Length of stomach (cm) Mean ± S.E.M	Distance covered (cm) Mean ± S.E.M	% Average travel	% Inhibition
1	Dist. Water(10ml/kg)	38.82 ± 3.02	27.44 ± 1.15	70.69	0.00
2	Diphenoxylate (5mg/kg)	36.97 ± 1.21	13.28 ± 0.33	35.93	64.07
3	AO (100 mg/kg)	34.73 ± 2.73	19.38 ± 0.97	55.83	44.17
4	AO (200 mg/kg)	44.07 ± 0.15	23.14 ± 1.56**	52.52	47.48
5	AO (400 mg/kg)	36.60 ± 1.01*	12.35 ± 0.61*	33.76	66.24

* indicate that values are significantly different from negative control, (P <0.05); ** indicate that values are significantly different from positive control, (P <0.01). AO means *Anacardium occidentale* leaf extract.

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shown to have dose related inhibitory actions on intestinal motility (Dicarlo *et al.*, 1994).

The presence of the flavonoid constituents present in *A. occidentale* may therefore be associated with the antidiarrhoeal findings.

Furthermore, findings from this study reveal the presence of antidiarrhoea principles in *A. occidentale* leaves, thus agreeing with traditional healers who use the fresh leaves in the management of diarrhoea. However, further studies to isolate and identify the active principle are encouraged.

TABLE III: Effect of *A. occidentale* extracts on castor oil-induced enteropooling in mice.

Group	Treatment	Weight of intestinal content (g) Mean ± S.E.M	Vol. of intestinal content (ml) Mean ± SEM
1	Dist. Water(10ml/kg)	0.43 ± 0.03	0.39 ± 0.03
2	Atropine (3mg/kg)	0.21 ± 0.04	0.23 ± 0.02**
3	AO (100 mg/kg)	0.41 ± 0.06*	0.26 ± 0.01
4	AO (200 mg/kg)	0.35 ± 0.02*	0. 22 ± 0.03
5	AO (400 mg/kg)	0.23 ± 0.08	0.14 ± 0.04

* indicate that values are significantly different from positive control, (P <0.01); ** indicate that values are significantly different from negative control, (P <0.05); AO: *Anacardium occidentale* leaf extract.

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