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EVALUATION OF ANTIDIARRHOEAL POTENTIAL OF METHANOLIC ROOT EXTRACT OF *Cassia sieberiana* DC (Fabaceae) IN MICE

Mohammed, Z[1]*. , Bello, H[2]. Yaro, A.H[3]., A. Ahmed[1]

[1]Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Biological sciences, Ahmadu Bello University, Zaria, Nigeria.

3 Department of Pharmacology and Clinical Pharmacy, Bayero University, Kano, Kano State, Nigeria.
* Corresponding Author: zeedullah1@yahoo.co.uk, 08033114092

Abstract

Cassia sieberiana (Fabaceae) is a tropical plant that has been reported to be traditionally used in Nigeria for the treatment of general body pain, ulcer, dysmenorrheal and diarrhoea. The phytochemical screening, toxicity evaluation and antidiarrhoeal activity studies of the methanolic extract of *C. sieberiana* were carried out. The anti-diarrhoeal activity was evaluated in mice using the experimental models of castor oil-induced diarrhoea, gastrointestinal motility test and castor oil-induced enteropooling in mice. A standard anti-diarrhoeal agent, loperamide, was used as reference drug. Phytochemically, the extract revealed the presence of flavonoids, terpenoids, tannins, saponins, glycosides and anthraquinones. The extract did not cause death nor show any sign of acute toxicity in the mice at the tested doses, thus implying that it was well tolerated by the mice. The different doses (100, 200 and 400 mg/kg) of the extract showed dose dependent and significant ($p<0.05$) decrease in the number of wet feaces in the castor oil-induced diarrhoea , inhibition of the propulsive activity of charcoal meal through the gastrointestinal lumen and reduction of intra-luminal accumulation in the castor oil induced enteropooling in mice. The antidiarrhoeal activity was comparable to that of loperamide at the higher dose of 400 mg/kg. It could be concluded from the results that *C. sieberiana* possess potent anti-diarrhoeal activity which validates its use as an antidiarrhoeal agent in traditional medicine practice.

Key words: *Cassia sieberiana*, castor oil, diarrhoea, loperamide, mice

Introduction

The majority of people living in the rural areas in developing countries live in deplorable and unhygienic conditions, which results in a lot of common diseases like diarrhoea. Herbal preparations are used in managing these various health conditions. Medicinal plants are indispensable component of traditional medicine practice worldwide because of the economic viability, easy accessibility and cultural acceptance. An estimated eighty percent of the world's population is reliant on traditional medicine, and the World Health Organization, WHO, has encouraged the use

of traditional medicine in the treatment and prevention of both acute and chronic diseases (Atta and Mouneir, 2004, WHO, 2008).

Diarrhoea is defined as the passage of three or more loose or liquid stools per day or more frequent passage than is normal for an individual (WHO, 1993). It is a symptom characterized by frequent passage of fluidly feaces, involving increase in peristaltic movement of the gastrointestinal tract, and also increase in secretions and a reduction in the absorption of fluids, thus resulting in water and electrolyte loss (Rang *et al*, 2006). Diarrhoea is caused by the alteration of the gastrointestinal tract function which is characterized by increased frequency of bowel sound and movement, wet stool and abdominal pain (Jafri, 2001; Ezekwensili *et al.*, 2004).Worldwide, it is one of the leading causes of death in children, especially those malnourished and the elderly due to dehydration associated with the disease and in Nigeria, it accounts as the number one killer among children aged 1-5 years (Audu *et al.*, 2000). Causative agents for diarrhoea include plant toxins, infectious agents, inflammatory disorders and dismotility problems affecting the gastrointestinal tract and substances which increase GIT secretion (Ezeigbo *et al.*, 2003). The WHO has constituted a diarrhoea disease control program (DDC) to enable studies of traditional medicine practices with the evaluation of health education and preventive approaches (Synder, 1982). Oral rehydration therapy (ORT) use has been widely identified as a key factor in the decline of diarrhoea (Robert *et al.*, 2006).

Synthetic antidiarrhoeal drugs caused side effects such as rashes, fever, nausea, vomiting, headache and joint pains (Soberon *et al.*, 2007). Based on this, many people have embarked on the use of indigenous plants as remedy against diarrhoea, and many plants have been reported to be used in treating and managing diarrhoea (Agunu *et al.*, 2005). Although several medicinal plants have become important in the treatment of diarrhoea, many are yet to be evaluated. Therefore, the search for safer and more effective agents has continued to be an important area of active research.

Cassia sieberiana belongs to the family Fabaceae. It is a tropical plant mostly consisting of shrub or small trees. It grows up to 5-20 m high, and has spirally arranged leaves with 5-14 pairs of leaflets; short dropping branches and has bright yellow flowers (Keay, 1989; Burkill, 1995). It is commonly called 'African drumstick tree' and has many vernacular names in Nigeria: it is known as '*Marga', 'Margaje', 'Kiskatigrai*' and '*Ifo*'among the Hausa, Fulani, Kanuri and Yoruba communities respectively.

Previous Phytochemical screening on the plant revealed the presence of flavonoids, anthracene derivatives, non-hydrolysable tannins, saponins and alkaloids (Modusolomuo *et al.*, 1999). Pharmacological studies on *C. sieberiana* established various uses; the roots extract is used traditionally to treat pain and inflammation, stem bark is used to treat

jaundice, haemorrhoids, bilharzias, leprosy, dropsy and bloody diarrhoea. The seeds are used as sedatives, purgative, while the leaves are used to treat burns, ulcers, rheumatism and diarrhoea and the whole plant extract is used to treat fever, inflammatory conditions, children's diseases and as vermifuge (Madusolumuo *et al.*, 1999; Neuwinger, 2000). The purpose of this present work is to evaluate the methanolic root extract of *C. sieberiana* for antidiarrhoeal activity to validate its use in traditional medicine practice for treating diarrhoea.

Methodology

Collection and identification of Plant materials

Fresh roots of *C. sieberiana* were collected in Giwa town, Giwa local government area of Kaduna State, Nigeria, in the month of April, 2010. The plant was taxonomically authenticated by Mal. U. Gallah of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Voucher specimen number (900202) has been deposited in the Department of Pharmacognosy and Drug Development of the University.

Preparation of Extracts

The roots of the plant were washed to remove dirt, sliced into small pieces and dried at room temperature for 2 weeks, then pulverized into coarse powder. Five hundred (500) g of powdered root was macerated using 20% water and 80% methanol with occasional shaking for 24 hours and filtered. The procedure was repeated twice for exhaustive extraction. The extract was concentrated using rotary evaporator. The percentage yield (w/w) was determined and the extract stored in the refrigerator for further use.

Experimental animals:

Locally bred adult Swiss albino mice (20-25g) of both sexes used were obtained from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were housed under standard conditions in stainless steel cages, had access to standard feeds (Pfizer Feeds, Nig. Plc) and access to clean drinking water *ad libitum*. The animals were acclimatized for 2 weeks before use and fasted over night with free access to water before the experiments. The study was conducted in accordance with the principles of laboratory animal care (NIH publication No. 85-23, revised 1985). All experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Drugs and chemicals

All chemicals and solvents used for the study were of analytical grade. The charcoal meal and castor oil (Well's Health Care, Spain) were purchased from Dialogue pharmacy and loperamide (Square Pharmaceuticals Ltd, Bangladesh) from Nana Pharmacy all in Kaduna, Kaduna state of Nigeria. Chemicals used such as normal saline (Orion Infusions Ltd, Bangladesh),

80% methanol (BDH Chemicals Ltd, Poole, England) were obtained from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.

Phytochemical screening of extract

The crude methanolic extract was tested for different phytochemical groups such as alkaloids, flavonoids; saponins, etc using standard methods (Evans, 2009; Sofowora, 2008; Harborne, 1991).

Acute Toxicity study

The median lethal dose (LD_{50}) of the methanolic extract of *C. sieberiana* root was determined using the method of Lorke (1983). In the first phase, nine animals were divided into 3 groups of 3 mice each and were given graded doses of 10, 100 and 1000 mg/kg body weight orally. The control group received 10ml/kg of distilled water. Animals were allowed free access to feed and water for 24 hours during which they were observed for signs of toxicity and death. In the second phase, 3 animals were divided into 1 mouse each. The animals were administered higher doses of 1000, 1600, 2900 and 5000 mg/kg body weight and then observed for 24 hours for sign of toxicity. The LD_{50} was calculated as the geometric mean of the minimum toxic dose and maximum tolerated dose in the second phase.

Evaluation of anti-diarrhoeal activity

Castor oil-induced diarrhoea

The method of Biswas *et al* (2002) as modified by Ezenkwesili *et al* (2004) was used for this experiment. The animals were fasted for 18 hours and were then randomly divided into 5 groups containing 6 animals each. Groups A, B,and C were administered orally with 100mg/kg, 200mg/kg and 400mg/kg respectively of root extract . Groups D and E received loperamide (5mg/kg, *p.o*) as positive control and distilled water (10ml/kg, *p.o*) as negative control. All animals were administered 0.5 ml of castor oil orally after one hour. After this administration, the animals were then placed separately in metal cages lined with transparent blotting paper which was changed every hour. The severity of diarrhoea was assessed each hour for 4 hrs. The total number of diarrhoeal feaces of control group was considered 100%. The result was expressed as a percentage of inhibition of diarrhoea (Zaval *et al.*, 1988). Percent inhibition of defecation in mice was calculated by using the following equation:

$$\% \text{ inhibition} = \left(\frac{\text{Mean defecation of control} - \text{Mean defecation of test sample}}{\text{Mean defecation of control}} \right)$$

Gastrointestinal Motility Test

Thirty mice were fasted for 18 hours but allowed access to water. They were then randomly divided into 5 groups of 6 mice each. Animals in groups A, B and C were treated orally with 100mg/kg, 200mg/kg and 400mg/kg respectively of the methanol extract of *C. sieberiana*. Groups D and E received loperamide (5mg/kg *p.o*) and

distilled water (10mg/kg *p.o*) respectively. Five minutes after the administration, 0.5 ml charcoal meal (10% of charcoal suspended in 5% acacia gum) was administered to each mouse using gastric intubation. After a period of thirty minutes, all the mice were sacrificed by cervical dislocation and the gastrointestinal tract (GIT) removed. The distance travelled by the charcoal from the pylorus was measured and expressed as percentage of the total length of small intestine extending from the gastropyloric to the ileocal junction (Madubuike and Onyeacholam, 2015). The percentage motility was calculated using the equation:

$$\% \text{ Motility } = \left(\frac{\text{Distance travelled by meal}}{\text{Total length of small intestine}} \right) \text{x}100$$

Effect of Extract on Castor Oil- Induced Enteropooling

This experiment was carried out using the method of Robert et al (1976) to determine intra-luminal fluid accumulation. Thirty mice fasted for 18 hours were randomly divided into 5 groups of 6 mice each. Root extract at doses of 100mg/kg, 200mg/kg and 400mg/kg were administered to animal in groups A, B and C respectively. Group D received loperamide (5mg/kg orally) while group E received distilled water (10mg/kg orally). After 1 hour, 0.5 ml of castor oil was administered orally to all animals. Two hours post treatment, all mice were sacrificed, the small intestine was removed after tying the ends with thread and the content of each intestine emptied into a graduated test tube and the volume recorded

Statistical Analysis

Results were presented as mean ± Standard Error of Mean (SEM). The data was statistically analyzed using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Dunnett's test. The results obtained were compared with the control group with p values < 0.05 considered to be statistically significant.

Result

Extraction

The methanol extract of *C. sieberiana* gave a dark brown coloured paste with pleasant smell. A yield of 7.4% w/w of dried extract was obtained.

Phytochemical Test

C. sieberiana root methanol extract revealed the presence of flavonoids, terpenoids, cardiac glycosides, tannins, phenols, saponins and anthraquinones.

Acute Toxicity

The median acute toxicity test (LD_{50}) was found to be above 5000mg/kg. Neither death nor any sign of toxicity was recorded.

Effect of the Extract on Castor Oil-Induced Diarrhoea

In the castor oil-induced diarrhoea experiment, the mice that did not receive the root extract showed typical diarrhoea signs like frequent and watery stooling. The onset of diarrhoea in the test groups at about 45

minutes after the castor oil administration was dose–dependent. The different doses of the extract (100, 200 and 400mg/kg) significantly ($p<0.05$) reduced the number of wet feaces in mice in comparison with the untreated animals in the control group. The effect at the highest dose of 400mg/kg was comparable to the standard anti diarrhoeal drug, loperamide (5 mg/kg) (Table 1). Diarrhoea was observed in mice in the control group 30 minutes after the administration of castor oil, and persisted throughout the duration of the experiment.

Effect of the Extract on charcoal Transit time

Results of the effect of *C. sieberiana* root extract on small intestinal transit are on Table 2. The results showed a dose–dependent reduction in all the doses (100, 200 and 400 mg/kg) of the extract on the coal meal through the GIT when compared to control. The activity was significant ($p<0.05$) and at the higher dose of 400 mg/kg, the activity was comparable to that of standard drug, loperamide (5mg/kg)

Table 1: Effect of methanol root extract of *C. sieberiana* on castor oil-induced diarrhoea in mice

Groups	Treatment with extract (mg/kg)	Mean number of wet feaces
A	100	10.0 ± 0.42*
B	200	9.2 ± 0.35*
C	400	7.0 ± 0.25*
D	Loperamide (5mg/kg)	6.6 ±0.58*
E.	Distilled water (10ml/kg)	18.5 ±1.03

*Values are expressed as mean ± SEM $p< 0.05$ when compared with negative control.

Table 2: Effect of methanol root extract of *C. sieberiana* on small intestine transit in mice

Group	Treatment with extract (mg/kg)	length of small Intestine (cm)	distance travelled by charcoal meal(cm)	% motility
A	100	39.65 ± 1.65	35.25±1.50	88.90*
B	200	43.08 ± 1.64	37.42±1.42	86.86*
C	400	39.40±1.25	26.32±1.60	60.13 *
D	Loperamide (5mg/kg)	41.04 ±1.30	23.10±1.20	58.63 *
E.	Distilled water (10ml/kg)	48.35± 1.98	45.40±2.10	93.89

* Values are expressed as mean ± SEM p< 0.05 when compared with negative control.

Table 3: Effect of methanol root extract of *C. sieberiana* on castor oil-induced entropooling in mice

Group	Treatment with extract (mg/kg)	Volume of content (ml)
A	100	0.19 ± 0.009*
B	200	0.17 ± 0.007*
C	400	0.07 ± 0.003*
D	Loperamide (5mg/kg)	0.06 ±0.005*
E	Distilled water10mg/kg)	0.32 ±0.013

*Values are expressed as mean ± SEM p< 0.05 when compared with negative control.

Effect of Extract on Castor oil-Induced Enteropooling

The effect of the extract of *C. sieberiana* on the castor oil-induced enteropooling showed a significant ($p< 0.05$) dose-dependent reduction of the intra-luminal fluid accumulation in the test animals when compared with those in the control group. The effect at the highest dose of 400mg/kg was comparable to that of loperamide (5mg/kg).

Discussion

Previous phytochemical screening of the extract revealed the presence of tannins, flavonoids, saponins, terpenoids, cardiac glycosides and anthraquinone. Some of the phytochemical constituents present in the root extract have been shown to have antidiarrhoeal activity. Antidiarrhoeal properties of medicinal plants have been found to be due to tannins, alkaloids, sterols, flavonoids and reducing sugars (Longanga *et al.*, 2000; Havagiray *et al*., 2004). Flavonoids have been established as inhibitors for the release of prostaglandins thereby reducing intestinal motility and secretion induced by castor oil (Dicarlo *et al.*, 1994; Palombo, 2005). The constituents present in the extract might be responsible for the antidiarrhoeal activity of *C. sieberiana* root observed.

The median acute toxicity study (LD_{50}) was carried out to determine any possible adverse reaction of the doses of the extract. Results revealed that the root extract at the tested doses produced neither death nor any sign of toxicity. The LD_{50} value obtained was above 5000mg/kg. This implied that the drug is well-tolerated by the animals because LD_{50} values of 5000mg/kg is considered safe (Lorke, 1983).

Diarrhoea is characterized by predominant secretory components and hypermotility of the gastrointestinal tract (Ezeigbo *et al.*, 2010). The antidiarrhoeal activity of *C. sieberiana* was evaluated using models such as castor oil induced diarrhea, charcoal meal transit time and castor oil enteropooling in mice (Havagiray *et al.*, 2004; Biwas *et al.*, 2002; Ezekwensili *et al.*, 2004). The results obtained in this work revealed that the methanolic extract of root of *C. sieberiana* significantly ($p< 0.05$) reduced the number of wet feaces caused by castor-oil in a dose-dependent manner. The castor oil used for inducing diarrhoea is enzymatically metabolized to ricinoleic acid in the small intestine thereby leading to changes in the permeability of mucosal cell layer resulting in the inflammation and irritation of the intestinal mucosa and diarrhoea. The degree of inhibition observed in the castor oil induced diarrhea by the extract could suggest a capacity in decreasing gastrointestinal tract motility and secretions which resulted in the observed antidiarrhoeal activity.

Previous studies have indicated the ability of activated charcoal to readily adsorb drugs and chemicals on the surface of the intestine, hence preventing absorption (Levy, 1982). The charcoal meal test was used to evaluate the effect of the plant extract on peristalsis movement. The extract and the reference

drug, loperamide significantly ($p<0.05$) reduced the intestinal motility and the fluid accumulation in the castor oil-induced enteropooling dose-dependently and was comparable to control at the higher dose (400 mg /kg). This was shown by the reduction in the intestinal length travelled by the charcoal meal in animals treated with the root extract when compared to the control. A reduction in the movement of the intestine and wet feaces are important considerations in diarrhoeal treatment. Most antidiarrhoeal drugs possess the ability of reducing intestinal contractions and consequently intestinal transit time (Bruton, 1996). *C. sieberiana* may be acting through the same mechanism. Loperamide used as reference drug possess antisecretory activity as well as cause inhibition of acetylcholine release resulting in the reduction of peristalsis in the GIT (Rang *et al.*, 2006).

The effect of the methanolic extract of *C. sieberiana* root on the castor oil induced enteropooling also showed that all doses of the extract significantly ($p<0.05$) reduced the intraluminal fluid accumulation in a dose dependent manner. The inhibition of intraluminal fluid secretion by the extracts in this study may be due to prostaglandin biosynthesis inhibition consequently resulting in decrease in secretion of fluid into the lumen. Suppression of intestinal fluid accumulation by the extract may be due to inhibition of gastrointestinal function (Nwafor *et al.*, 2000).

Conclusions

In conclusion, results obtained in this study indicated that the methanolic root extract of *C. sieberiana* possess antidiarrhoeal activity which was comparable to that of reference drug, loperamide. Thus, the results provide the rationale for the use of *C.sieberiana* root in traditional medicine practice in Nigeria for treating diarrhoea. Further studies are however necessary to isolate, purify and establish the identity of the active constituents in the crude extract of *C. sieberiana* responsible for the antidiarrhoeal activity and the possible mechanism of action.

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