Journal of Advances in Biology & Biotechnology
3(4): 139-144, 2015; Article no.JABB.2015.041
ISSN: 2394-1081

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Studies on Anti-diarrheal Activity of *Cassia sieberiana* in Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Author MKG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OOF managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2015/13810
Editor(s):
(1) Joana Chiang, Department of Medical Laboratory Science and Biotechnology, China Medical University, Taiwan.
Reviewers:
(1) J. Daniel Dubreuil, Département de Pathologie et Microbiologie, Université de Montréal, Canada.
(2) Maitreyee R Brahmbhatt, Pharmacognosy, Gujarat Technological University, India.
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(4) Peter G. Mwitari, Kenya Medical Research Institute, Kenya.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=931&id=39&aid=9189

Original Research Article

Received 5th September 2014
Accepted 27th April 2015
Published 8th May 2015

ABSTRACT

Purpose: *Cassia sieberiana* (*Fabaceae*), is one plant that has been reported to possess numerous pharmacological activities. The purpose of this study is to scientifically investigate its anti-diarrheal property.
Methods: The anti-diarrheal activity of the methanol extract of *Cassia sieberiana* leaves was evaluated in mice following various experimental models: Castor oil – induced diarrhea, gastrointestinal motility test and castor oil – induced enteropooling. A standard anti-diarrheal agent, loperamide was used as reference.
Results: Different doses (100, 200 and 400 mg/kg, *p.o.*) of the extract significantly ($p < 0.05$) decreased the number of wet fecal droppings in the castor oil – induced diarrhea, and also inhibited the propulsion of charcoal meal plug through the gastrointestinal lumen. All doses of the extract also significantly ($p < 0.05$) reduced the castor oil induced enteropooling. The actions of the extract were dose – related and comparable to the actions of loperamide, the standard drug. The extract did not evoke any sign of acute toxicity in mice at the doses tested; thus it was well tolerated by the mice.

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Conclusion: The results suggest that *C. sieberiana* possess potent anti-diarrheal effect which validates its use as a non – specific regimen for treating diarrhea in traditional medicine.

Keywords: Cassia sieberiana; extract; diarrhea; castor oil; loperamide; mice.

1. INTRODUCTION

Diarrhea is defined as the frequent passage of fluidy feces, involving an increase in the peristaltic movement of the gastrointestinal tract. It also include an increase in secretions and a reduction in the absorption of fluid, resulting in electrolytes and water loss [1]. Causes of diarrhea include infectious agents, plant toxins, gastrointestinal disorders such as inflammatory and dismotility problems of gastrointestinal tract and substances that increase GIT secretions [2]. In developing countries, the majority of people living in rural areas use herbal preparations in managing various health conditions including diarrhea, which is one of the major causes of death in children especially the malnourished. [3].

Cassia sieberiana is native to Africa and belongs to the family *Fabaceae*. It grows 10 – 20 m in height, and has flowers that are bright yellow in color. The leaves are arranged in leaflets that contain 7 – 10 pairs of opposite leaves [4]. The roots of the plant are used as a diuretic and vermifuge in treating diseases like elephantiasis, diarrhea, leprosy, dysentery, hemorrhoids and venereal diseases. The seeds are used as sedatives while the leaves are used to relieve symptoms of arthritis and rheumatism [5].

In an *In vitro* evaluation test, different extracts (benzene, methanol and water) of the leaves of *C. sieberiana* showed antimalarial efficacy against *Plasmodium falciparium* [6]. This study however reports on the anti-diarrheal effects of *C. sieberiana* using various models of experimentally induced diarrhea.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Cassia sieberiana* were collected in June (during the rainy season) from Nsukka, Enugu State, Nigeria. The Plant was identified by the Botany Department, Michael Okpara University of Agriculture, Umudike, Nigeria. A voucher specimen is deposited in our herbarium for further reference.

2.2 Preparation of Extract

The fresh leaves were dried under mild sunlight, then pulverized into coarse powder. Five hundred (500) grams of the sample were macerated in solvent (80% methanol and 20% water) for 48 hours with intermittent shaking. Filtration was done using filter papers and funnel into an already weighed beaker. The filtrate was concentrated using rotary evaporator and later lyophilized. The percentage yield (w/w) was determined and the extract was stored in the refrigerator (4°C) throughout the period of the study.

2.3 Animals

Swiss albino mice (25 – 35 g) were used for the study. The animals were kept in stainless steel cages at room temperature (23°C – 27°C) and relative humidity of about 45 - 65% and fed *ad libitum* with standard commercial pelleted feed. Clean drinking water were provided and the animals were allowed 2 weeks for acclimatization before the experiments. Ethical rules guiding the use of laboratory animals for experiments according to Zimmerman [7] were strictly followed.

2.4 Preliminary Phytochemical Analysis of the Extract

The tests were carried out according to the procedures outlined by Harbourne [8], Trease and Evans [9].

2.5 Acute Toxicity Test

Twenty five mice were randomly divided into 5 groups of 5 mice each. The test groups received varying doses of extract (500 mg/kg, 1000 mg/kg, 2000 mg/kg and 3000 mg/kg *p.o*), while the control group received 10 ml/kg of distilled water. The animals were allowed free access to feed and water for 48 hours during which they were observed for signs of toxicity and death.

2.6 Castor Oil-induced Diarrhea

The method of Biswas et al. [10] as modified by Ezekwesili et al. [11] was used. Mice fasted for

18 h were randomly divided into 5 groups of 6 mice each. Groups A, B and C were treated orally with 100 mg/kg, 200 mg/kg and 400 mg/kg respectively of the methanol leaf extract of *C. sieberiana*. Group D received loperamide (5 mg/kg, *p.o.*) while group E received distilled water (10 ml/kg, *p.o.*) and served as the negative control. After 1 h, 0.5 ml of castor oil was orally administered to each animal placed in a separate cage. Transparent blotting papers were placed beneath each cage and the animals were observed for a period of 4 h for the presence of wet feces.

2.7 Gastrointestinal Motility Test

The effect *C. sieberiana*. of on charcoal meal transit time was evaluated using the method of Mascola et al. [12], modified by Chidume et al. [13]. Thirty mice were fasted for 18 h but allowed free access to clean water. They were randomly divided into 5 groups of 6 mice each. Groups A, B and C were treated orally with 100 mg/kg, 200 mg/kg and 400 mg/kg respectively of the methanol leaf extract of *C. sieberiana*. Group D received the standard drug loperamide (5 mg/kg, *p.o.*) while group E received distilled water (10 mg/kg, p.o.). Five minutes after drug administration, 0.5 ml charcoal meal (10% charcoal suspension in 5% acacia gum) was administered to each mouse by gastric intubation. Thirty minutes later all the animals were sacrificed by cervical dislocation and the gastrointestinal tract removed. The distance travelled by the charcoal meal from the pylorus was measured and expressed as a percentage of the total length of the small intestine, extending from the gastropyloric to the ileocecal junction. The percentage motility was derived from the equation:

$$\% \text{ Motility} = \frac{\text{Distance travelled by the meal}}{\text{Total length of the small intestine}} \times \frac{100}{1}$$

2.8 Castor Oil – Induced Enteropooling

Intra-luminal fluid accumulation was determined by the method of Robert et al. [14]. Thirty mice fasted for 18 h were randomly divided into 5 groups of 6 animals each. Groups A, B and C received 100, 200 and 400 mg/kg of the extract respectively, by gastric intubation. Group D received loperamide (5 mg/kg orally) while group E received distilled water (10 ml/kg, *p.o.*). 1 h later, 0.5 ml of castor oil was orally administered to all the animals. Two hours post treatment, all the mice were sacrificed, the small intestine was removed after tying the ends with thread. The content of each intestine was milked into a graduated test tube and the volume recorded.

2.9 Statistical Analysis

The experimental results are presented as mean±SEM (standard error of the mean) and analyzed using one way analysis of variance (ANOVA). The difference between the means was tested using the Post Hoc LSD and values of $p < 0.05$ were considered significant in the study.

3. RESULTS

3.1 Extraction

Extraction of *C. sieberiana* with methanol resulted in a yield of 12.6% w/w dry matter. The extract was very sticky and dark greenish in color.

3.2 Preliminary Phytochemical Test

A preliminary phytochemical examination of the methanol extract of *C. sieberiana* leaves revealed the presence of flavonoids, saponins, tannins, phenols and anthraquinones.

3.3 Acute Toxicity

The acute toxicity test recorded neither death nor sign of acute toxicity, thus the LD50 could not be determined.

3.4 Effect of the Extract on Castor Oil – Induced Diarrhoea

Thirty minutes following administration of castor oil, diarrhea was noticed in all the mice of the negative control group and it persisted throughout the duration of the experiment. In the test groups diarrhea started about 45 minutes after administration of castor oil. In a dose – related manner, various dose (100, 200 and 400 mg/kg) of the extract evoked significant ($p < 0.05$) reduction of the number of wet feces when compared with the untreated control mice. However the effect of the extract even at the highest dose (400 mg/kg) tested was significantly ($p < 0.05$) lower than that of loperamide (5 mg/kg), the standard anti-diarrheal agent (Table 1).

3.5 Effect of the Extract on Small Intestinal Transit

The result of *C. sieberiana* on small intestinal transit is shown in Table 2. The result revealed that all the doses (100, 200 and 400 mg/kg) of the extract significantly ($p < 0.05$) reduced the movement of the charcoal meal through the gastrointestinal tract. The activity was dose - dependent but significantly ($p < 0.05$) lower than that of loperamide (5 mg/kg).

3.6 Effect of the Extract on Castor Oil – Induced Enteropooling

Studies on the castor oil – induced enteropooling showed that all doses of the extract significantly ($p < 0.05$) reduced the intra-luminal fluid accumulation in the test animals, when compared with the negative control animals (Table 3).

4. DISCUSSION AND CONCLUSION

An imbalance between the secretory and absorptive mechanisms in the intestinal tract could result in diarrhea, which manifest as frequent voiding of watery feces. Hypermotility usually characterize the type of diarrhea in which the secretory componenet dominates [3]. In the small intestine the castor oil utilized for the induction of diarrhea in the study is metabolized to ricinoleic acid. This causes irritation and inflammation of the intestinal mucosa resulting in the release of prostaglandins which stimulates intestinal motility and secretions [3,15]. The primary action of the reference drug (loperamide) is activation of presynaptic opioid receptors located in the enteric nervous system – an action that results in inhibition of acetylcholine release and reduction of peristaltic movement [1]. In addition to its antimotility action, loperamide also possesses antisecretory activity [1]. These pharmacological actions justify the use of loperamide as a reference anti-diarrheal drug in the study.

The results of this work reveal that the methanolic leaf extract of *C. sieberiana* evoked a significant ($p < 0.05$) reduction in the number of wet fecal droppings caused by castor oil. Also *C. sieberiana* was able to reduce intestinal motility significantly ($p < 0.05$) as evidenced by the significant decrease in the intestinal length travelled by the charcoal meal in the treated animals compared to the control. Reduction of intestinal motility and decrease in voiding of wet feces could be important actions in the treatment of diarrhea [15]. The acute toxicity study was carried out in order to determine possible adverse reactions to a single dose or an overdose of the extract [2]. It should be observed that the extract, at the doses tested produced neither death nor acute toxicity sign, indicating that the extract was well tolerated by the animals.

Table 1. Effect of methanol leaf extract of *C. sieberiana* on castor oil – induced diarrhea in mice

Group	Treatment	Mean number of wet faeces
A	Extract (100 mg/kg)	9.00±0.35*
B	Extract (200 mg/kg)	8.21±0.24*
C	Extract (400 mg/kg)	7.14±0.46*
D	Loperamide (5 mg/kg)	5.22±0.23*
E	Distilled water (10 ml/kg)	15.25±1.02

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

Table 2. Effect of methanol leaf extract of *C. sieberiana* on small intestinal transit in mice

Group	Treatment	Length of small intestine (cm)	Dist. travelled by charcoal meal (cm)	% motility
A	Extract (100 mg/kg)	40.08±1.64	31.34±1.36	78.19*
B	Extract (200 mg/kg)	42.02±1.78	31.28±1.41	74.44*
C	Extract (400 mg/kg)	39.70±1.29	28.22±1.52	71.08*
D	Loperamide (5 mg/kg)	41.26±1.81	23.34±1.29	49.30*
E	Distilled water (10 ml/kg)	46.24±1.97	43. 24±2.47	93.51

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

Table 3. Effect of methanol leaf extract of *C. sieberiana* on castor oil – induced enteropooling in mice

Group	Treatment	Volume of intestinal content (ml)
A	Extract (100 mg/kg)	0.16±0.008*
B	Extract (200 mg/kg)	0.15±0.007*
C	Extract (400 mg/kg)	0.13±0.007*
D	Loperamide (5 mg/kg)	0.09±0.004*
E	Distilled water (10 ml/kg)	0.24±0.011

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

Previous reports have demonstrated anti-diarrheal activity of tannins, flavonoids, and saponins, - containing plant extracts [16]. Flavonoids are also known for inhibiting release of prostaglandins, thereby limiting intestinal secretion and motility induced by castor oil. These constituents, which were found to be present in *C. sieberiana* may be responsible for the anti-diarrheal actions of the methanolic leaf extract of *C. sieberiana.*

The results could indicate that *Cassia sieberiana.* possesses significant anti-diarrheal activity, which justifies its use as an anti-diarrheal agent in Nigerian traditional medicine. Further studies are required to isolate the anti-diarrheal principle and also unravel its mechanism of action.

ETHICAL APPROVAL

The authors declare that this work was not against public interest. Animal experiments were conducted in accordance with NIH guidelines for care and use of Laboratory animals (Pub. No. 85 – 23, Revised 1985). Approval for this work was granted by the Ethical Committee, Department of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture, Umudike. The approval number is: MOUAU/VPP/EC/146.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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