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Original Article

Antidiarrhoeal Effects of Methanol Root Extract of *Detarium microcarpum* (Leguminosae) in albino Rats

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ABSTRACT

This study aimed at evaluating the antidiarrhoeal effects of methanolic root extract of *Detarium microcarpum*. The root of *Detarium microcarpum* was collected air-dried, ground to powder and extracted with methanol by cold extraction method to give a percentage yield of 9.65% w/w. The antidiarrhoeal effect of the methanol root extract on castor oil-induced diarrhoea, intestinal charcoal meal transit and castor oil-induced enteropooling were determined. The root extract of *Detarium microcarpum* produced a significant dose-dependent protection ($p<0.05$) against the castor oil-induced diarrhoea with the highest protection of 76.7% obtained at the highest dose tested (400mg/kg). The extract showed a significant intestinal charcoal meal transit ($p<0.05$) as it had 45.5%, 53.8%, and 62.4% inhibition respectively when compared to distilled water, the negative control (0% inhibition). Atropine however produced a significant increase ($p<0.05$) in intestinal charcoal meal transit at 78.1% inhibition. There were no significant differences ($p>0.05$) between the extract doses 100, 200, 400 mg/kg administered. The methanol extract at 100, 200, and 400 mg/kg showed 25.4%, 40.7% and 78.8% fluid accumulation respectively. The positive control Atropine (5 mg/kg) had 82.3% inhibition of intestinal content when compared to the negative control (distilled water treated rats) which had 0% inhibition. The results obtained from this study revealed that the root extract of *Detarium microcarpum* possesses antidiarrhoeal effect. The plant extract is recommended for bioassay-guided isolation and characterization of the active compounds responsible for the antidiarrhoeal property.

Keywords: Antidiarrhoea, *Detarium microcarpum,* Methanol root extract, Castor oil-induced diarrhoea

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1. INTRODUCTION

The use of medicinal plants in the treatment of diseases has generated renewed interest in recent times, as herbal preparations are increasingly being used in human healthcare systems. Diarrhoea is one of the common clinical signs of gastrointestinal disorders caused by both infectious and non-infectious agents. Diarrhoea in particular remains a major concern in Nigeria resulting in high mortality rates when left untreated. Traditional medicine remains the only health care available in many rural areas in Nigeria like the rural community of Borno state, located in a very remote area in the Northeastern part of the country. With limited access to modern medicine, the local population uses medicinal plants to treat most diseases.

Medicinal plants are those plants whose chemical contents have some physiological effect on the body chemistry. From the earliest times, mankind has used plants in an attempt to cure disease and relieve physical suffering. [1]. The folkloric use of plants to treat ailments has triggered the interest of researchers in tapping the valuable and enormous treasure packed in plants' nature. The therapeutic value of medicinal plants is due to substances found in the plant tissue that produce a definite physiological action on the human body. Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the backbone of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences. [1].

Medicinal plants play a golden role not only as a traditional medicine but also as trade commodities, meeting the demand of distant markets for the development of new drugs. In fact, to realize the effective integration of plants into a medical system, researchers and practitioners should be trained in both modern and traditional medicine in the use of plant compounds. In addition, to build credibility for the use of plants in conventional medicine, the empirical arguments should be converted into evidence-based arguments [2].

Detarium microcarpum Guill and *perr*. (leguminosae) is well- known wild edible fruit species growing in Sahara and sub-Sahara countries and is found mostly in savannah forests of dried type.[3]. *microcarpum* is a small straight trunk tree of 8-10 m high with a spherical fairly dense top. The leaves are alternate, imparipinnate with 3-6 pairs of alternate or sub-opposed, oval oblong or elliptic leaflets with rounded apex. The petal flowers have 4 sepals and 8-10 prominent cream-white stamens. The fruits are an ovoid or globular drupe, more or less flattered 2.5-5 cm in diameter, with a cracked surface at maturity, containing a large central nucleus surrounded by a greenish. Fibrous and sweet farinaceous pulp [3].

The leaves, flowers, fruits and seeds of *D. microcarpum* are used in human food. The fruits are rich in vitamin c and can be eaten raw or cooked, leaves and flowers are used as condiments and vegetables for the preparation of sauces. Leaves, bark, roots, fruits and seed of *D. microcarpum* are also used as a pharmacopoeia for the treatment of stomach pain, dysentery, malaria, jaundice, furunculosis, panaris, mining and sexually transmitted disease. The leaves, roots and trunk bark of *D. microcarpum* are used in Burkina Faso by the sanan in the treatment of infections and infestations, musculoskeletal, skin, digestive, nutritional and pregnancy disorders [3].

Inspite of the use of *D. microcarpum* in traditional medicine it is pertinent therefore to test the methanol extract on diarrhoea given the fact that microorganisms are becoming increasingly resistant to synthetic agents. The increase in the search for the phytotherapeutic

chemical constituents in plant materials has been remarkably astonishing, especially in recent times. Scientific justification of the folkloric use of *D. microcarpum* as anti-diarrhoea would help to provide information for the basis of the use of this plant for medicinal purposes.

2. MATERIALS AND METHODS

Equipment's used for pharmacological studies were: rodents' plastic cages, surgical gloves, face masks, 1- and 2-ml syringes, beakers, and surgical blades. Instruments used for pharmacological studies were: analytical weighing balance. Chemicals used for pharmacological studies were distilled water (Dona Nig. Ltd), castor oil (Bellsons and Co. Ltd, England), 5 % activated charcoal (Kochlight Laboratories Ltd. England), loperamide (RPG Life Science Ltd, Anleshwar), Atropine (Fugisawa U.S.A Inc).

Sample Collection, Identification and Preparation

Fresh sample root of *Detarium microcarpum* was collected from Sangere in Girei Local government Adamawa state, Nigeria and authenticated by Plant taxonomy. The sample was dried under shade rendered free of foreign material through manual picking and grounded with a wooden mortar and pestle to a fine powder.

Plant Extraction

Three thousand grams (3000g) of the pulverized dried root of *Detarium microcarpum* was macerated with absolute methanol at 25^{o}C for seventy-two hours (72hr) in a round bottom flask with occasional shaking. The soaked sample was passed through a muslin cloth to remove the vegetative debris and the liquid was then filtered through Whatman No 1 filter paper. The crude extract was concentrated to dryness at 25^{o}C. The extract was weighed, labelled and subjected to further analysis. 200g of the crude extract was subjected to defatting with n-hexane and then partitioning with chloroform, ethyl acetate and n-butanol base on their polarities using the dry technique.

Experimental Animals

Ninety (90) Wistar albino rats weighing between 100-200g were brought from Jos, Plateau State. They were kept in plastic rat cages and allowed to acclimatize to the laboratory environment for a minimum period of one week before the commencement of the experiments. They were fed with grower's mash (Vital Feed Nig Ltd, Jos, Nigeria) and water was provided *ad libitum.* The experiments were conducted in compliance with the international guiding principles for biochemical research involving animals [4]

Antidiarrhoeal Studies of *Detarium microcarpum*

Effect of the Methanol Root Extract of *Detarium microcarpum* on Castor Oil-Induced Diarrhoea

The method of [6] and adopted by [1] was used to evaluate the effect of the methanol extract on castor oil induced-diarrhoea. Thirty (30) Wistar strain albino rats of both sexes weighing between 100-200 g were used for the experiment. The rats were denied food for 12 hours but were provided with water *ad libitum*. They were divided into five groups of six rats each. Groups B, C and D were dosed orally with 100mg/kg 200 mg/kg and 400mg/kg of methanol crude extract respectively. Group A was given 2 ml of normal saline (Dona Nig. Ltd) orally. Group E was given 3mg/kgloperamide (RPG Life Science Ltd, Ankleshwar) intraperitoneally (*i.p*) as the standard drug. The rats were separated singly in cages lined with white blotting paper. After one hour, each rat was given 1ml castor oil (Bellsons and Co. Ltd, England), orally and observed for 6 hours for wet or watery faeces. The wet faeces of each rat were counted and recorded at the end of the experiment. The percentage protection was calculated using the formula below (Equ. 1) [6,7,] and adopted by [1].

$$\% \text{ protection} = \frac{A-B \times 100}{A} \qquad \text{(Equ. 1)}$$

where
A=mean defecation of the control group
B= mean defecation of the treated group

Effect of the Methanol Root Extract of *Detarium microcarpum* on Gastro Intestinal Transit of Charcoal Meal in Rats

The method of [5,8] and adopted by [1] was used to study the effect of the methanol root extract of *Detarium microcarpum* on gastrointestinal transit of charcoal meal in rats was calculated. Thirty (30) albino rats

weighing between 100 to 200 g were used for this experiment. The rats were denied food for eighteen hours but were allowed access to water *ad libitum*. They were divided into five groups of six rats each. Group A was served as the control and was given 2 ml of normal saline orally. Rats of groups B, C and D were given 100mg/kg, 200 mg/kg and 400mg/kg of methanol crude extract respectively. Group E rats were treated with 3mg/kg atropine (Fugisawa U.S.A Inc) intraperitoneally as the standard drug. After 10 minutes, 1 ml of charcoal meal (5% activated charcoal (Kochlight Laboratories Ltd. England) suspension in 10 % solution of acacia powder) was given orally to each rat. The rats were sacrificed after 30 minutes and the abdomen opened. The distance travelled by the charcoal meal was measured and expressed as a percentage of the total length of the intestine. The percentage of intestinal transit of the charcoal meal was calculated using the formula (Equ. 2).

% Intestinal Transit = X/Y*100 (Equ. 2)

Where
X=movement of charcoal (cm)
Y=Total length of the intestine (cm)

A reduction in the gastrointestinal propulsion of the charcoal meal was an indication of Anti-diarrhoeal effect [9; 7] and [1] while an increase in the gastrointestinal propulsion was an indication of a laxative effect.

Effect of the Methanol Root Extract of *Detarium microcarpum* on Castor Oil-Induced Enteropooling

The method of [10] and adopted by [1] used to evaluate the effect of the methanol root extract *Detarium microcarpum* on the intraluminal fluid accumulation in rats. Thirty (30) albino rats weighing between 100 to 200g were used for this experiment. The rats were fasted overnight and then divided into five groups of six rats each. Group A was served as the control and was given 2 ml normal saline per body weight orally. Groups B, C and D were given 100mg/kg, 200 mg/kg and 400mg/kg orally of the methanol crude extract respectively. Group E was treated with 3 mg/kg Atropine intraperitoneally (*i.p.*) to serve as the control drug. After one hour, all the rats were sacrificed and the intestine was removed and weighed. The content of the intestine was collected by milking and the weight of the empty intestine and the content was also measured.

Statistical Analysis

Data where applicable was presented as mean ± standard deviation (S.D). One way analysis of variance (ANOVA) was used to test for significance and mean and $P<0.05$ was considered significant.

3. RESULTS

Antidiarrhoeal Activity of the Extract of *Detarium microcarpum.*

Effect of Methanol Root Extract of *Detarium microcarpum* on castor oil-induced Diarrhoea

After one hour of administration of castor oil, to the rats the diarrhoea was clinically apparent in all the animals in the control group, for the next six hours. This was reduced by loperamide (3 mg/kg) [94.5 %]. A similar reduction in the number of defecations after six hours was achieved with the root extract of *Detarium microcarpum* at the doses of 100, 200 and 400 mg/kg. The methanol root crude extract of *D. microcarpum* at 100, 200 and 400 mg/kg significantly inhibited the defecation (47.9%, 65.7% and 76.7%) respectively. The effect of methanol root extract of *D. microcarpum* at (400 mg/kg) was comparable to that of loperamide, the standard anti-diarrhoeal agent at a dose 3 mg/kg concentration (94.5 %). There was a no significant difference between the effect of the extract doses ($p>0.05$), however, there was a significant difference ($p<0.05$) between the effect of the positive control, 3 mg/kg Loperamide when compared to the extract doses of 100 mg/kg, 200 mg/kg and 400 mg/kg on the total number of stools and wet stool as presented in Table 1.

Table 1: Effect of Extract on Castor Oil-Induced Diarrhoea

S.N.	Group	Dosage (mg/kg)	Mean ± SEM total no. of wet stool	% Inhibition
1	A	-ve control (saline)	14.60 ± 1.67 ^{c}	00
2	B	100	7.60 ± 1.54 ^{ab}	47.9
3	C	200	5.00 ± 1.05 ^{a}	65.7
4	D	400	3.40 ± 0.87 ^{a}	76.7
5	E	3 (Loperamide)	0.80 ± 0.20 ^{ad}	94.5

Means with different letters are significantly different from each other ($p<0.05$) in the column

Effect of Methanol Root Extract of *Detarium microcarpum* on Gastrointestinal Transit of Charcoal Meal in Rats

The methanol root extract of *Detarium microcarpum* 100 mg/kg, 200 mg/kg and 400 mg/kg dose of extract produced 45.5%, 53.8% and 62.4% of gastrointestinal transit induced by castor oil respectively. Atropine however produced a significant decrease ($p<0.05$) in percentage intestinal transit (78.1%). There was no significant difference ($p>0.05$) between the methanol root crude extract of *Detarium microcarpum* doses 100, 200 and 400 mg/kg administered. However, there was significant difference ($p<0.05$) between the effects of the methanol root crude extract of *Detarium microcarpum* when compared to the negative control (rats pretreated with distal water) and positive control (rats treated with atropine) as presented in Table 2.

Table 2: Effect of Extract on Intestinal Transit in Rats

S.N.	Group	Dosage (mg/kg)	Mean± SEM Total length of Intestine (cm)	Mean ± SEM Movement of charcoal (cm)	% Intestinal Transition	% Inhibition
1	A	-ve control (saline)	83.60±2.23 ^{a}	65.00±1.64 ^{c}	77.7	00
2	B	100	80.00±1.84 ^{a}	35.40±6.19 ^{ab}	44.2	45.5
3	C	200	75.80±1.91 ^{a}	30.00±5.70 ^{a}	39.5	53.8
4	D	400	77.80±2.06 ^{a}	24.40±1.63 ^{a}	31.3	62.4
5	E	3 (Atropine)	72.40±4.43^{a}	14.20±0.86 ^{ad}	19.6	78.1

Mean with the same letters no significant difference ($p> 0.05$) while mean with different letters across the column are significant different from each other ($p< 0.05$)

Effect of Root Extract of *Detarium microcarpum* on Castor Oil-Induced Entropooling

Castor oil-induced entropooling is not influenced by atropine 3 mg/kg in rats. Methanol extract of *Detarium microcarpum* 100, 200 and 400 mg/kg produce a dose dependent reduction in intestinal weight and volume. The methanol root extract of *Detarium microcarpum* 100, 200 and 400 mg/kg dose produced 25.4 %, 40.7 %, 78.8 % and 82.3 % inhibition of the volume of intestinal content respectively with significance ($p<0.05$). The weight of intestinal content was also reduced significantly at all the doses Table 3.

Table 3: Effect of Extract on Castor oil-Induced Entropooling

S. N.	Group	Dose (mg/kg)	Mean ± SEM Weight of intestine and content (g)	Mean ± SEM Weight of empty intestine (g)	Mean ± SEM Weight of content (g)	% fluid Accumu-lation	% inhibi-tion
1	A	-ve control (saline)	5.04±0.03	2.88±0.22 ^{ab}	2.56±0.15	50.6	00
2	B		5.81±0.29 ^{ab}	4.37±0.52 ^{a}	1.90±0.01 *	32.7	25.4
3	C		4.79±0.24 ^{a}	3.28±0.24	1.51±0.12 *	31.5	40.7
4	D		4.41±0.34 ^{b}	3.84±0.23	0.54±0.13 **	12.2	78.8
5	E		5.09±0.07	4.50±0.06 ^{b}	0.46±0.03 **	8.8	82.3

Mean with the same a steric across the column no significant difference ($p > 0.05$) while mean with different letters across the column are significantly different from each other ($p < 0.05$)

4. DISCUSSION

Diarrhoea (loose motions) is the passage of 3 or more loss of liquid stools per day or more frequently than is normal for the individual. Diarrhoea is not itself a disease, but can be a symptom of several diseases and sometimes may be associated with abdominal pain, which may reduce after a stool is passed. Diarrhoea occurs due to the irritation within the lining of the small or large intestine, which leads decrease water absorption and hence increases in water being passed with stools. Many factors such as food poisoning, infection (bacterial, viral, parasitic), food intolerance malnutrition, intestinal disease and sometimes medication can contribute to diarrhoea. Castor oil has been reported to induce diarrhoea by increasing the volume of intestinal contents and preventing the re-absorption of water [1]. The plant extract at doses of MCE 100, 200 and 400 mg/kg significantly decreased ($p < 0.05$) the total number of wet faeces produced upon administration of castor oil at 400 mg/kg was comparable to the control group. The effect of the highest dose of the MCE was similar to that of the standard drug. Therefore, it may probably assume that the anti-diarrhoeal action of the extracts was mediated by an anti-secretory mechanism. These include castor oil decrease fluid absorption, increases secretion in the small intestine and colon and effects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of recinolic acid [1]. The propulsion of the charcoal meal through the gastrointestinal tract decreased significantly ($p < 0.05$) from MCE at 100, 200 and 400 mg/kg, compared to the control group. Similarly, the effect of the highest dose of the MCE of the graded dose was similar to that of the standard drug. These observations suggest that the extracts reduced diarrhoea by inhibiting peristalsis, gastrointestinal motility and castor oil-induced enteropooling. It may be equally effective in the prevention and curing of diarrhoea. The significant inhibition of the castor oil-induced enteropooling in rats suggests that the extract of *Detarium microcarpum* produced relief in diarrhoea by spasmolytic activity in vivo and anti-enteropooling effect [1, 11].

5. CONCLUSION

The methanol root extract of *Detarium microcarpum* has been shown to possess some remarkable anti-diarrhoeal activity from different models used in this study. It is recommended that the bioassay-guided isolation of the bioactive compound(s) responsible for the anti-diarrhoeal activity and characterization of the active compounds be carried out.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

7. ACKNOWLEDGEMENTS

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