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EXPERIMENTAL PAPER

Antidiarrheal activity of fractions from aqueous extract of *Detarium senegalense*

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Summary

The aim of this study was to evaluate the effect of different fractions of the aqueous crude extract of *Detarium senegalense* stem bark on castor oil-induced diarrhea. Castor oil-induced diarrhea, gastrointestinal motility and castor oil-induced enteropooling methods were used to evaluate the antidiarrheal effects of the fractions. Castor oil was used to induce diarrhea and the effect of all the fractions (chloroform, ethyl acetate, *n*-butanol, methanol and residual aqueous) were evaluated at the doses of 200 and 400 mg/kg body weight. The results show that all fractions significantly ($p<0.05$) decreased the frequency of defecation in rats following the induction of diarrhea with castor oil. Ethyl acetate which produced the highest antidiarrheal activity was further subjected to gastrointestinal motility and castor oil-induced enteropooling tests. In the gastrointestinal motility, two

test doses of the extract (200 and 400 mg/kg) were administered orally to two groups of rats (n=5), while the third group of rats (control), were treated with normal saline, and the fourth group were treated with diphenoxylate, a conventional anti-diarrheal drug. In the castor oil-induced enteropooling experiment, normal saline was used for the control animals, while 200 and 400 mg/kg of the extract was administered to groups two and three, respectively and atropine, a standard drug, was administered to rats in group four. The ethyl acetate fraction significantly ($p<0.05$) decreased the gastro-intestinal motility, as shown by the extent of movement of the charcoal meal in the treated rats. It also significantly inhibited the fluid accumulation within the intestine. These findings suggest that the ethyl acetate fraction possess antidiarrheal effect, which may be due to the presence of some phytochemical constituents (alkaloids, flavonoids and tannins) in the plant, which may either be working alone or in combination with each other. This study has demonstrated that *D. senegalense* fractions, especially the ethyl acetate fraction, possess antidiarrheal activity thus supporting the use of the plant in the treatment of diarrheal diseases.

Key words: *Detarium senegalense, stem bark extract, antidiarrheal activity*

INTRODUCTION

Detarium senegalense JF Gmelin (family: *Leguminoseae*) is a plant found mostly in west and central Africa along water course [1].

The plant is regarded as a veritable source of natural medicine. The inhabitants of these regions have been using the decoctions from various parts of this plant for treatment of a plethora of both animals and human diseases. The stem bark and/or leaves are used in folk medicine as an anti-diarrhea in Nigeria and other West African countries [2, 3]. It is also employed for the treatment of pneumonia, anaemia, and expulsion of the placenta after child birth. In an earlier study, the aqueous stem bark extract of *D. senegalense* was observed to have anti-diarrheal effect in treated rats [4]. The active phytochemical compounds isolated from *D. senegalense* include cyclohexanone, ß-myrcene, cis-rose oxide camphor and citronellol from the petroleum ether extracts of the seed [3].

In Africa, particularly in Nigeria, herbal medicine has become a part of the people's culture with about 70% depending mainly on traditional medicine [5, 6], due to high cost of conventional primary health service.

Diarrheal diseases cause almost three million deaths per year globally, mainly among under-five children [7]. It is the major cause of morbidity and mortality in infants in the developing countries [8]. The cost of conventional antidiarrheal drugs in these areas is high, coupled with their non-availability, thus raising the need for an alternative remedy available locally for the treatment of this condition.

The aim of the present study was to further determine the phytochemical constituents and the antidiarrheal activity of different fractions of *D. senegalense* on albino rats. The results of the antidiarrheal study would identify the most active organic solvent fraction of *D. senegalense* stem bark and give validity to its possible use as an antidiarrheal.

MATERIALS AND METHODS

Sample collection and identification

Fresh samples of the leaves and stem bark of *D. senegalense* were collected in April, 2009 from Marama, Biu Local Government Area, Borno State, Nigeria. The plant was identified and authenticated by Dr. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Nigeria, where a voucher specimen (Biological science 178[A]) was deposited.

Preparation of extract

Fresh stem bark of collected *D. senegalense* were air-dried in a shade, ground into fine powder and stored in a glass container at 4°C. One hundred grams of the powdered sample was exhaustively extracted with distilled water using a reflux method. The crude aqueous extract obtained was evaporated to dryness in hot air oven at 40–50°C; the extract was brown in colour and yielded 33.21% (w/w). It was properly labeled and stored in the refrigerator at 4°C until used [9].

Fractionation of the aqueous extract

The crude aqueous extract obtained was suspended in cool distilled water and then filtered using Whatman No. 1 filter paper. The filtrate was thereafter successfully fractioned with chloroform, ethyl acetate, *n*-butanol and methanol. The fractionation with the organic solvents of different polarity was performed until the organic layer was visibly clear to obtain chloroform, ethyl acetate and *n*-butanol, in sequence, as described in earlier studies [10, 11]. The residual aqueous fraction at this point was evaporated to dryness and the dried residue extracted with methanol to obtain methanol soluble fractions and residual aqueous fraction. All fractions were evaporated to dryness and reconstituted in distilled water before administration.

Phytochemical analysis

The *D. senegalense* organic solvent fractions were subjected to qualitative chemical screening for identification of the various classes of active chemical constituents such as carbohydrates, tannins, phlobatannins, saponins, steroids, flavonoids, alkaloids, anthraquinones, and triterpennoids. The phytochemical analyzes were done by standard methods [12, 13].

Experimental animals

Adult Wistar albino rats of both sexes (110–205 g) used in the study were acquired from the animal unit of the Department of Biochemistry, University of Maiduguri, Maiduguri, Borno State, Nigeria. The animals were fed with standard feed (Vital Feeds, Jos, Nigeria), provided with free access to water under a well-ventilated condition of 12 hrs. light cycle. They were kept in plastic cages and were allowed to acclimatize for two weeks before the commencement of the experiments. The study was carried out in accordance with the Organization for Economic and Development (OECD) principles on Good Laboratory Practice (GLP) [14]. Prior ethical approval (Code number, UMSE/06/012) was obtained from the ethical committee on the use of animals of the College of Medical Sciences, University of Maiduguri, Nigeria.

Effects of organic solvent fractions on castor oil-induced diarrhea

Sixty rats of both sexes weighing between 112–161 g were used for this study. The castor oil test was performed as described by Offiah and Chikwendu [15]. The rats were divided into twelve groups of five animals each. They were housed singly in cages lined with white blotting paper. The animals in groups 1 and 2 were treated with the chloroform extract of *D. senegalense* at 200 and 400 mg/kg, respectively. Those in groups 3 and 4 were treated with ethyl acetate extract fraction at 200 and 400 mg/kg, respectively. Groups 5 and 6 rats received *n*-butanol extract fraction at 200 and 400 mg/kg. Methanol extract fraction was given to the rats in groups 7 and 8 at 200 and 400 mg/kg, respectively. The rats in groups 9 and 10 were treated at the doses of 200 and 400 mg/kg, respectively, with residual aqueous fraction of the crude extract. The animals in group 11 received 5 mg/kg of diphenoxylate (Searl, Germany) intraperitoneally, while the rats in group 12 were given 2 ml/kg normal saline (Dana, Nigeria). One millilitre of castor oil was given to each rat 1 h after pretreatment of the rats with organic solvent extracts and the drug. The extracts and the castor oil were administered orally. The animals were kept and observed over a period of 6 h for the frequency of passing watery (wet) or unformed stool. Absence of such watery dropping was recorded as a positive, indicating protection.

Effect of ethyl acetate fraction on gastrointestinal motility

The method of Akter *et al*. [16], was used to test the effect of *D. senegalense* ethyl acetate fraction on the gastrointestinal motility of rats. The albino rats (4 groups, n=5) were starved of food but given free access to water for 18 h before extract or drug administration. The rats in group 1 were treated with 2 ml/kg of

normal saline (Dana, Nigeria) orally; those in groups 2 and 3 received ethyl acetate fraction of *D. senegalense* orally at a dose of 200 and 400 mg/kg, respectively, while rats in group 4 were administered 3 mg/kg of atropine sulphate intraperitoneally. About 1 h later, 1 ml of 5% charcoal suspension in 10% aqueous solution of *Acacia* powder was given orally to each rat. After 30 min, the animals were sacrificed by cervical dislocation. The small intestine of each rat was carefully examined and removed. The distance travelled by the marker (charcoal) from the pylorus was measured and expressed as percentage of total length of the intestine from the pylorus to the caecum.

Effect of the ethyl acetate fraction on castor oil-induced enteropooling

The intraluminal fluid accumulation test was conducted according to the method of Robert *et al*. [17]. The animals (5 rats/group) weighing 150–185 g were fasted over night before pretreatment with 2 ml/kg of normal saline orally, and 200 and 400 mg/kg of ethyl acetate fraction orally for the rats in groups 1, 2 and 3, respectively. The rats in group 4 were pretreated with 3 mg/kg of atropine, intraperitoneally. One hour later, 1 ml of castor oil was orally administered to each rat. At the expiration of another 1 h, the rats in each group were sacrificed, the small intestine of each rat removed, its content collected and weighted.

STATISTICAL ANALYSIS

All data were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA), with Tukey-Kramer multiple comparisons test were used to detect differences between groups. Values lower than 0.05 were considered significant.

RESULTS

The results of the qualitative phytochemical analyzes of the organic solvent fractions of aqueous extract of *D. senegalense* indicated the presence of carbohydrates, tannins, saponins, glycosides, flavonoids, alkaloids and terpenes/steroids in the various organic solvent fractions, while phlobatannins and anthraquinones were absent.

Castor oil produced copious diarrhea in all the rats within 2–3 h after its administration. The administration of the different fractions at the doses of 200 and 400 mg/kg orally to rats as well as the standard drug diphenoxylate significantly ($p<0.05$) decreased the frequency of defecation in rat following the induction of diarrhea with castor oil.

The ethyl acetate fraction at the two doses used protected the rats from diarrhea induced by castor oil more than the other organic solvent extract fractions used in the present study. The rats in the control group defecated averagely about 20 times within the 6 h period of observation. The animals in groups 3 and 4 that received 200 and 400 mg/kg of ethyl acetate fraction of the extract had a mean frequency defecation of 6.67±1.20 and 5.67±1.12 times, respectively; those treated with diphenoxylate did not defecate within the 6 h observatory period that is indicating 100% protection (tab. 1).

Table 1.

Effect of *D. senegalense* stem bark organic solvent and water residue fractions on castor oil-induced diarrhea in rats

Treatment groups	Extract dose [mg/kg]	Mean number of defecation in 6 hours	Percent protection [%]
1. Chloroform + CO	200	11.67±2.07*	43.50±1.04*
2. Chloroform + CO	400	10.67±2.32*	47.50±1.08*
3. Ethyl acetate + CO	200	6.67±1.20*	67.81±1.02*
4. Ethyl acetate + CO	400	5.67±1.12*	72.35±0.05*
5. *n*-Butanol + CO	200	10.00±2.30*	51.12±1.03*
6. *n*-Butanol + CO	400	12.00±1.98*	41.94±1.06*
7. Methanol + CO	200	8.67±2.20*	57.73±1.04*
8. Methanol + CO	400	8.67±2.20*	58.02±1.08*
9. Water residue + CO	200	10.67±2.32*	48.04±0.81*
10. Water residue + CO	400	9.00±1.75*	55.92±0.02*
11. Control saline + CO	–	20.67±2.57	–
12. Diphenoxylate + CO	5	0.00±0.00*	100

Value mean ±SD based on five observations,
* – significant ($p<0.05$) decrease compared to control,
CO – castor oil.

There was also a significant ($p<0.05$) difference in the small intestine transit time of the charcoal between control and groups treated with ethyl acetate fractions and the standard drug atropine. The charcoal moved very rapidly along the small intestine in the control group, while the movement reduced markedly in the rats treated with the 200 and 400 mg/kg ethyl acetate doses. The rats treated with atropine (3 mg/kg) had the shortest distance of charcoal movement along the small intestine compared to the other groups (tab. 2). The intestinal transit of charcoal in the groups treated with 200 and 400 mg/kg doses of ethyl acetate fraction of aqueous extract appeared to be statistically similar with that of atropine.

Table 2.

Effect of *D. senegalense* stem bark ethyl acetate fraction on gastrointestinal transit of charcoal in rats

Treatment groups	Total length of intestine [cm]	Distance travelled by charcoal [cm]	Percent of intestinal transit [%]
Control (saline)	107.87 ± 2.35	90.73 ± 1.21	85.04 ± 2.81
Extract (200mg/kg)	100.50 ± 1.74	51.67 ± 2.15*	50.93 ± 1.21*
Extract (400mg/kg)	103.10 ± 2.55	47.83 ± 2.32*	47.09 ± 0.27*
Atropine (3mg/kg)	104.07 ± 3.15	18.30 ± 2.55*	18.24 ± 0.23*

Value mean ± SD based on five observations.
* – significant ($p<0.05$) decrease compared to control

In the castor oil-induced intestinal fluid accumulation test, there was a significant ($p<0.05$) increase in intestinal content weight of the control as compared to groups treated with ethyl acetate fraction (tab. 3). The percent fluid accumulation for the control group was 31.33 ± 1.24%, while those of the groups treated with 200 and 400 mg/kg of ethyl acetate extract fraction were 5.03 ± 0.51% and 4.31 ± 1.32%, respectively. The group treated with atropine had the least intestinal content weight, with fluid accumulation of 2.57 ± 0.62%.

Table 3.

Effect of *D. senegalense* stem bark ethyl acetate fraction on induced enteropooling in rats

Treatment groups	Weight of intestineand content [g]	Weight of empty intestine [g]	Difference in weight [g]	Percent intestinal transit [%]
Control (saline)	5.80 ± 0.42	4.02 ± 0.52	1.78 ± 0.19	31.33 ± 1.24
Extract (200 mg/kg)	5.27 ± 0.26	4.98 ± 0.27	0.28 ± 0.04*	5.03 ± 0.51*
Extract (400 mg/kg)	5.52 ± 0.30	5.28 ± 0.30	0.24 ± 0.01*	4.31 ± 1.32*
Atropine (3 mg/kg)	5.15 ± 0.24	5.02 ± 0.23	0.13 ± 0.02*	2.57 ± 0.62*

Value mean ± SD based on five observations,
* – significant ($p<0.05$) decrease compared to control.

DISCUSSION

The results of the present study have demonstrated that the organic solvent fractions of aqueous extract of *D. senegalense* stem bark possess antidiarrheal activities in castor oil treated rats. The extracts of the various organic solvent

fractions protected against diarrhea induced by castor oil. Castor oil contains ricinoleic acid which when liberated induced irritation of the gastro-intestinal mucosa resulting in inflammation, increased secretion of fluid and electrolytes and increased motility of the gastrointestinal tract resulting in diarrhea [18]. Castor oil is also thought to induce diarrhea by releasing prostaglandin from the colonic cells [19, 20].

Reports have indicated that *D. senegalense* possess anti-inflammatory properties [2, 21]. It is possible that the anti-diarrhea action exerted by these extracts may be related to the inhibition of prostaglandin formation. However, confirmation through further studies is needed before such an assertion. Umukoro and Ashorobi [22] reported that the seed extract of *Aframomum melegueta* inhibited diarrhea induced by castor oil in rodents through prostaglandin dependent mechanism. Diarrhea also may occur due to decreased reabsorption of substances within the intestine [23]. Diphenoxylate, an opioid substance, is known to inhibit gastrointestinal secretion and motility [18].

In this study, the ethyl acetate fraction of the aqueous extract of *D. senegalense* was observed to decrease significantly ($p<0.05$) the intestinal transit period of charcoal and reduce the intestinal content weight of rats. The decrease in gastrointestinal motility will allow the intestinal contents to be exposed to the absorptive surface of the intestinal tract for longer time [24]. The effect of the extract in this respect may be similar to that of atropine a standard drug used in this study. Atropine is a known antimuscarinic agent [25] which inhibits gastrointestinal motility.

The organic solvent fractions of the aqueous extract of *D. senegalense* contain, amongst others, alkaloids, tannins and flavonoids. Alkaloids have been known to have analgesic, anti-inflammatory and antidiarrheal effects [26]. Furthermore, tannins are known to have astringent properties and therefore could be used to treat diarrhea [27]. The protein precipitated by tannins cover the surface of the cell or tissue, acting as a barrier between the irritant and the tissue, with underlying tissue protected from damage. This process could reduce intestinal mucous membrane secretions [28]. Flavonoids have been demonstrated to ameliorate contractions induced by spasmogens [29, 30]. Thus, the chemical constituents of the extract may contribute to its antidiarrheal properties. This finding is in agreement with the claim of herbal healers who use the plant to treat diarrhea [3].

CONCLUSION

In conclusion, the results of the current study have shown that *D. senegalense* stem bark organic solvent fractions of the aqueous extract possess anti-diarrhea effect, and have provided evidence that support the traditional use of the plant extract in the treatment of diarrheal diseases in both animals and man.

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DZIAŁANIE PRZECIWBIEGUNKOWE RÓŻNYCH FRAKCJI WYCIĄGU WODNEGO Z *DETARIUM SENEGALENSE*

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Streszczenie

Celem niniejszej pracy było określenie wpływu różnych frakcji surowego wyciągu wodnego z kory pnia *Detarium senegalense* na hamowanie biegunki indukowanej olejem rycynowym u szczurów. Działanie przeciwbiegunkowe tych frakcji, po wywołaniu biegunki olejem rycynowym, określono na podstawie obserwacji ruchów robaczkowych jelit oraz

gromadzenia płynu w świetle jelita. Badano działanie przeciwbiegunkowe różnych frakcji (chloroformowej, n-butanolowej, metanolowej oraz otrzymanej za pomocą octanu etylu, jak i pozostałości wodnej) w dawkach 200 i 400 mg/kg masy ciała szczura. Badania wykazały, że wszystkie frakcje istotnie ($p<0.05$) obniżyły częstość defekacji u szczurów po wywołaniu biegunki w stosowanym modelu. Frakcja otrzymana przez działanie octanem etylu, wykazująca najsilniejsze działanie przeciwbiegunkowe, została następnie użyta w ocenie ruchów robaczkowych jelita i gromadzenia płynów w jego świetle. W teście oceny ruchów robaczkowych jelit dwóm grupom szczurów (n=5) podawano dwie dawki wyciągu (200 i 400 mg/kg), trzecia kontrolna grupa szczurów była traktowana izotonicznym roztworem soli, natomiast czwarta difenoksylatem jako standardowym lekiem przeciwbiegunkowym. W teście oceny gromadzenia płynów w świetle jelita grupie kontrolnej podano izotoniczny roztwór soli, grupie drugiej i trzeciej podano odpowiednio 200 i 400 mg/kg wyciągu, natomiast czwartej grupie podano atropinę jako lek standardowy. Stwierdzono, że frakcja otrzymana za pomocą octanu etylu istotnie ($p<0.05$) spowolniła ruchy robaczkowe jelit, co zaobserwowano jako stopień hamowania efektu wywołanego podaniem węgla drzewnego szczurom. Frakcja ta również istotnie hamowała gromadzenie płynu w jelicie. Otrzymane rezultaty sugerują, że frakcja otrzymana za pomocą octanu etylu ma właściwości przeciwbiegunkowe, prawdopodobnie ze względu na obecność w roślinie aktywnych związków chemicznych (alkaloidów, flawonoidów i tanin), które mogą działać bądź pojedynczo lub jako ich kombinacja. Podsumowując wykazano, że frakcje z *D. senegalense*, a szczególnie frakcja otrzymana za pomocą octanu etylu, mają właściwości przeciwbiegunkowe, co może wskazywać na możliwość zastosowania badanej rośliny do leczenia chorób przebiegających z biegunką.

Słowa kluczowe: *Detarium senegalense, wyciąg z kory pnia, właściwości przeciwbiegunkowe*