Journal of Experimental Pharmacology

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ORIGINAL RESEARCH

Antidiarrheal activity of methanolic extract of the root bark of *Cordia africana*

This article was published in the following Dove Press journal:
Journal of Experimental Pharmacology
17 October 2016
Number of times this article has been viewed

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Abstract: An ethnobotanical study in Agew-Awi and Amhara peoples in northwest Ethiopia reported that *Cordia africana* is used traditionally in the treatment of liver disease, amebiasis, stomachache, and diarrhea. The root and root bark are reported to be used in the treatment of diarrhea. Therefore, this study was intended to evaluate the antidiarrheal effect of *C. africana* against castor oil-induced diarrhea in mice. The antidiarrheal effect of the plant was tested on castor oil-induced diarrhea in mice (23–25 g) of either sex. Number of diarrheic defecations, intestinal length traveled by the charcoal meal, and weight of intestinal fluid were taken as important parameters to evaluate the antidiarrheal activity of the plant extract. In preliminary phytochemical screening tests, the methanolic extract of *C. africana* was found to contain phenols, flavonoids, terpenoids, and saponins. Reduction in the number of diarrheic drops was observed in groups of mice that received 200 mg/kg ($P<0.05$) and 400 mg/kg ($P<0.01$) of the extract compared to the negative controls. The percent inhibition of intestinal fluid accumulation was 26.83%, 46.34%, and 53.66% at the doses of 100, 200, and 400 mg/kg of the extract, respectively. Relative to the negative control group, the mean percent of intestinal length moved by the charcoal meal was decreased by 24.41%, 39.89%, and 51.66% in groups of mice given 100, 200, and 400 mg/kg of the plant extract, respectively. To iterate the finding, the root bark extract of *C. africana* was found to be effective in preventing castor oil-induced diarrhea and intestinal motility in a dose-dependent manner. This reveals that the plant material has promising antidiarrheal activity as it is claimed in traditional medical practice.

Keywords: *Cordia africana*, antidiarrheal activity, castor oil, traditional medicine, mice

Introduction

Diarrhea is the passage of loose or watery stools at least 3 times in a 24-hour period and classified as acute and chronic mainly based on the duration of symptoms. Acute diarrhea is usually caused by bacteria (eg, *Campylobacter*, *Salmonella*, *Shigella*, and *Escherichia coli*) and viruses (eg, rotavirus). It is also caused by medications like antibiotics, anticancer drugs, and antacids containing magnesium. On the other hand, chronic diarrhea is usually related to a functional disorder such as irritable bowel syndrome or an intestinal disease such as Crohn's disease. Additionally, parasitic (*Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium*) infection may also be a cause for chronic or persistent diarrhea.[1]

Diarrhea is one of the major health problems worldwide, especially for children under 5 years of age, and it is more problematic in developing countries. Diarrhea remains the second-leading cause of death among children under 5 years of age globally. About 1.5 million children die each year due to diarrhea.[2] The burden of diarrheal illness affects more the developing world, in terms of both morbidity and mortality.[1]

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http://dx.doi.org/10.2147/JEP.S116155

Majority of people living in developing countries rely on traditional medicine to treat different ailments including diarrhea.[3] It is estimated by the World Health Organization that around 80% of the population in Africa use traditional medicines and about 85% of traditional medicine involves the use of plant extracts.[4]

In an ethnobotanical survey conducted in northwest Ethiopia, different parts of *Cordia africana* (small to medium-sized tree, belonging to the family Boraginaceae, native to Ethiopia) have been reported to be used for different ailments such as liver disease, amebiasis, stomachache, and diarrhea.[5] Similarly, several ethnobotanical studies reported that many plant species belonging to the family Boraginaceae such as *Cordia myxa*, *Cordia rothii*, *Cordia americana,* and *Carmona retusa* have therapeutic use in diarrhea in different societies.[6–9] In addition, a mere traditional claim, *Onosma bracteatum,* belonging to the Boraginaceae family, has been demonstrated to have valuable antidiarrheal activity in vivo.[10]

Previous phytochemical studies on *C. africana* revealed that the plant material contains coumarins, saponins, tannins, triterpenes, and flavonoids,[11] and these secondary plant metabolites have confirmed antidiarrheal effects.[12] Furthermore, the in vitro studies of *C. africana* demonstrated promising antioxidant, anti-inflammatory, antibacterial, and cytotoxic activities and the authors support the use of *C. africana* leaves in traditional medicine to treat inflammation-related conditions and infectious diseases.[13,14] Besides, plant materials with antioxidant and cytotoxic activities are claimed to be effective in managing oxidative stress disorders and disorders that may complicate oxidative stress like diarrhea.[15,16]

Lack of pharmacologic activity screening report for the claimed traditional use of the root bark of *C. africana* in treating diarrhea and all the aforementioned evidences were considered to justify the practicability of this study. Accordingly, the purpose of this study was to evaluate the antidiarrheal activity of the crude methanolic extract of root bark of *C. africana* in experimental animals.

Materials and methods

Drugs, chemicals, and materials

Drugs and chemicals that were used for this study include loperamide (batch no: F1216001; Bafna Pharmaceuticals Ltd, Chennai, India), atropine (Laboratoire Renaudin, Itxassou, France), activated charcoal (batch no: Eox/5809/ 1903/13; Lab Reagent, Mumbai, India), castor oil (batch no: OA-052; Jordan Amman Pharmaceutical Industries), and methanol (lab chemicals; Hyderabad, India). Other laboratory chemicals and reagents were also used in phytochemical screening test.

Experimental animals

Swiss albino mice (23–25 g) of either sex bred in the animal house of Department of Pharmacology, University of Gondar were used for the experiment. The mice were housed in cages and maintained under standard conditions (room temperature and humidity, 12-hour light and dark cycle). Standard pelletized food and tap water were provided ad libitum.

Plant material

The root bark of *C. africana* was collected from Chilga district, which is 810 km away from the capital city of Ethiopia, Addis Ababa, in the northwest direction and 50 km away from Gondar town to the west-northwest Ethiopia. In the meantime, the fresh plant part (leaf) was brought and sent to Addis Ababa for authentication and the plant specimen was deposited in the National Department of Herbarium, Department of Biology, Science Faculty, Addis Ababa University (voucher No: AB002).

Preparation of the plant extract

C. africana root bark was dried in shade and cut into small pieces. Then, 300 g of this plant material was extracted with adequate amount of 80% methanol by macerating in a beaker for 24 hours. The first extract was filtered using Whatman filter paper (grade no: 101), and additional solvent was used for the second round extraction of the residue for the same length of time and was filtered in the same fashion. Then, the extract was dried in an oven adjusted to 40°C and stored in refrigerator until use for the intended test.[12]

Phytochemical chemical screening test

Preliminary phytochemical chemical screening tests were carried out on the methanolic extract of *C. africana* according to the methods mentioned by Trease and Evans[17] and Jones and Kinghorn.[18]

Acute toxicity test

Acute toxicity study was conducted for the crude extract (single dose 2,000 mg/kg) using the OECD guideline for the testing of chemicals. Five female Swiss albino mice weighing 20–25 g were used. Each mouse received the extracts at a dose of 2,000 mg/kg body weight. Signs of toxicity were observed for the first 2 hours and in 2-hour intervals for 6 hours. Finally, the number of survivors was noted after 24 hours.[19,20]

Castor oil-induced diarrhea in mice

Initially, all the animals were screened and selected for the experiment if they were found diarrheic when they were

exposed to 0.2 mL of castor oil before 5 days of the actual experiment. After being fasted for 3 hours, the 30 mice were divided into five groups of six mice in each group. The first group received distilled water, 2 mL/100 g body weight (negative control), while the second group received loperamide, 3 mg/kg body weight (positive control). Three serial double doses of the plant extract, 100, 200, and 400 mg/kg body weight, were administered orally to groups 3, 4, and 5, respectively. The doses were determined based on acute toxicity test results. One hour later, all the animals were given 0.2 mL of castor oil orally. Then, the animals were kept in separate metabolic cages and the severity of diarrhea was assessed each hour for 4 hours. The total score of diarrheal feces for the control group was considered as 100%. The results are expressed as a percentage of inhibition of diarrhea relative to the negative control group.[21]

Castor oil-induced enteropooling in mice

Thirty mice were randomly selected and fasted for 18 hours. Just before the procedure, the mice were divided into five groups of six animals in each group. Thereafter, group 1 animals were given distilled water orally (2 mL/100 g body weight) (negative control). Group 2 animals received loperamide, 3 mg/kg body weight, orally. Groups 3, 4, and 5 were given 100, 200, and 400 mg/kg serial double doses of the plant extract, respectively, orally. Immediately afterwards, castor oil (0.2 mL) was administered to all the mice. Thirty minutes later, the mice were sacrificed and the small intestine of each mouse from the pylorus to the caecum was isolated and the isolated intestine was weighed immediately. Then, the intestinal content of each mouse was removed by milking and the empty intestine was reweighed. The difference between the first and the second weights was considered as the weight of the intestinal content of each mouse.[22]

Gastrointestinal motility in mice

Thirty mice were randomly selected and starved for 24 hours prior to the experiments, but free access of water was allowed. Just before the procedure, the mice were randomly divided into five groups, each containing six mice. Groups 1 and 2 were given 2 mL/100 g distilled water and 1 mg/kg atropine sulfate, respectively. Animals in groups 3, 4, and 5 received 100, 200, and 400 mg/kg serial doses of the crude extract. Five minutes after drug administration, 0.5 mL of a 5% charcoal suspension in distilled water was administered to each animal orally. Then, each animal was sacrificed 30 minutes later and the abdomen was opened. The percentage distance of the small intestine travelled by the charcoal plug was determined.[23]

Data analysis

The difference between means of the same parameter was determined by one-way analysis of variance followed by Dunnett's post hoc test using SPSS version 16. At 95% confidence interval ($P<0.05$), the result was considered as statistically significant.

Ethical clearance

The animals were handled according to guidelines for the housing of mice in scientific institutions,[24] and the ethical clearance was requested and obtained from the Experimental Animals Ethics Committee, Department of Pharmacology, University of Gondar.

Results

Phytochemical chemical screening test

Preliminary phytochemical chemical screening tests on methanolic extracts of the root bark of *C. africana* showed the presence of phenols, flavonoids, terpenoids, and saponins (Table 1).

Preliminary acute toxicity test

Oral administration of the methanolic root bark extract of *C. africana* at a dose of 2,000 mg/kg body weight did not produce any mortality and remarkable signs of toxicity.

Table 1 Results of identification tests of secondary metabolites in root bark of *C. africana*

Secondary metabolites	Method used for identification	Reagents used	Result
Phenols	Ferric chloride test	Ferric chloride solution	Positive
Anthraquinones	Borntrager's test	Benzene, 10% ammonia	Negative
Flavonoids	Lead acetate test	Lead acetate solution	Positive
Phytosterols	Salkowski reaction	Petroleum ether, chloroform, concentrated H_2SO_4	Negative
Alkaloids	Mayer's test	1% HCl, Mayer's reagent	Negative
Glycosides	Modified Borntrager's test	Dilute hydrochloric acid, ferric chloride, benzene	Negative
Terpenoids	Salkowski test	Chloroform, concentrated H_2SO_4	Positive
Saponins	Foam test	Distilled water	Positive

Abbreviation: *C. africana*, *Cordia africana*.

Castor oil-induced diarrhea

Compared to the negative control, the three serial doses (100, 200, and 400 mg/kg) of the extract reduced the total number of fecal output significantly (P<0.001) in 4 hours of observation. The mean number of defecation for the negative control group was 11.83±0.79, while in the extract-treated group this value was found to be 6.60±0.40, 5.75±1.03, and 5.17±0.54 at the doses 100, 200, and 400 mg/kg body weight, respectively, and 1.50±0.22 for the positive control group. Loperamide showed higher degree of reduction than all of the three serial doses of the extracts (P<0.001).

Statistically significant reduction in the number of diarrheic drops was observed in groups of mice that received 200 and 400 mg/kg of the extract. However, the reduction was to a lesser extent compared to the reduction by loperamide. From a control value of 5.67±0.92, diarrhea score was significantly reduced to 3.00±0.45 (P<0.05), 2.20±0.37 (P<0.01), and 0.50±0.22 (P<0.001) by the extract at 200 mg/kg, 400 mg/kg and loperamide (3 mg/kg) respectively, with 47.09%, 61.20%, and 91.18% protection of diarrhea in order. The degree of reduction in diarrheal drops by 400 mg/kg of the extract was significantly higher (P<0.01) than the reduction by 100 mg/kg of the extract (Table 2).

Castor oil-induced enteropooling

C. africana root bark extract decreased castor oil-induced enteropooling significantly in mice at doses of 200 (P<0.01) and 400 mg/kg (P<0.001) of body weight. The mean intestinal fluid accumulation in the negative control group was 0.41±0.05 g, 0.22±0.01 g in the group of mice that received 200 mg/kg of the extract, and 0.19±0.01 g in the group of mice that received 400 mg/kg of the extract. The percentage inhibition of intestinal fluid accumulation was 26.83%, 46.34%, and 53.66% at the doses of 100, 200, and 400 mg/kg of the extract, respectively. The standard drug, loperamide (3 mg/kg), reduced (0.12±0.01 g, P<0.001) intestinal fluid accumulation by 70.73%. Even though there was still statistically significant difference (P<0.05), the 200 and 400 mg/kg doses of the extract decreased the intestinal fluid accumulation to closer degree of reduction seen by loperamide. The highest dose of the extract (400 mg/kg) showed significantly greater reduction in the intestinal fluid accumulation compared to the lowest dose of the extract (100 mg/kg), but showed no significant difference between the effects of the highest dose and the middle dose (200 mg/kg) of the extract (Table 3).

Intestinal transit of charcoal meal

Compared to the value for the negative control group (79.02%±4.85%), the three consecutive doses of the extract significantly reduced the percent mean of the small intestine transit by charcoal meal in a dose-dependent manner. The percent transit of charcoal meal in the group of mice given 100 mg/kg of the extract was 59.73±3.10 (P<0.05), group

Table 2 Effect of methanolic extract of *C. africana* on castor oil-induced diarrhea in mice

Groups	Dose (mg/kg)	Onset of diarrhea (minutes)	Total number of feces	Total number of diarrheic drops	Weight of fresh fecal output (g)	Inhibition of defecation (%)#	Inhibition of diarrheal drops (%)#
Group 1 (water), negative control	–	25.00±1.39	11.83±0.79	5.67±0.92	1.21±0.17	0	0
Group 2 (loperamide), positive control	3	129.83±4.80^{a,***}	1.50±0.22^{a***}	0.50±0.22^{a***}	0.25±0.08^{a***}	87.32	91.18
Group 3 (extract)	100	25.83±1.56^{b,***}	6.60±0.40^{a***,b***}	4.50±0.29^{b***}	1.04±0.08^{b***}	44.21	20.63
Group 4 (extract)	200	40.17±5.13^{a,**,b,***,c,**}	5.75±1.03^{a***,b***}	3.00±0.45^{a*,b*}	0.83±0.24^{a**,b***}	51.39	47.09
Group 5 (extract)	400	96.20±14.51^{a***,b***,c***,d***}	5.17±0.54^{a***,b***}	2.20±0.37^{a**,b*,c**}	0.47±0.05^{a**,c***,d**}	56.30	61.20

Notes: Values are in mean ± SEM (n=6). [a]Compared with negative control; [b]compared with positive control; [c]compared with group 3; [d]compared with group 4. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001. #% Inhibition is relative to the negative control group.
Abbreviations: *C. africana*, *Cordia africana*; +ve, positive; –ve, negative; SEM, standard error of mean.

Table 3 Effect of methanolic extract of *C. africana* on intestinal fluid accumulation in mice

Groups	Dose (mg/kg)	Weight of intestinal fluid (g)	Inhibition of weight of intestinal content (%)#
Group 1 (water), negative control	–	0.41±0.05	0
Group 2 (loperamide), positive control	1	0.12±0.01^{a***}	70.73
Group 3 (extract)	100	0.30±0.02^{b***}	26.83
Group 4 (extract)	200	0.22±0.01^{a**,b*,c}	46.34
Group 5 (extract)	400	0.19±0.01^{a***,b*,c**}	53.66

Notes: Values are in mean ± SEM (n=6). [a]Compared with negative control; [b]compared with positive control; [c]ompared with group 3. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001. #Inhibition (%) is relative to the negative control group.
Abbreviations: *C. africana*, *Cordia africana*; SEM, standard error of mean.

of mice given 200 mg/kg of the extract was 47.50±5.96 (P<0.001), and group of mice given 400 mg/kg of the extract was 38.20±2.34 (P<0.001). The mean percent of intestinal transit of the charcoal meal was much more markedly reduced by atropine (26.48±2.68%, P<0.001). Relative to the negative control group, the mean percent of intestinal length moved by the charcoal meal was decreased by 24.41%, 39.89%, and 51.66% in groups of mice given 100, 200, and 400 mg/kg of the plant extract, respectively. In mice given atropine (1 mg/kg), this value was 66.49%. There is a significant difference between the effects of different doses of the extract. The highest dose showed more degree of reduction in intestinal transit compared to the lowest (P<0.001) and the middle (P<0.05) doses of the extract. Compared to atropine, the three serial doses of the plant extract showed smaller degree of reduction in intestinal transit of the charcoal meal (Table 4).

Discussion

In an ethnobotanical study, *C. africana* is reported to be used in the management of diarrhea in Shinasha, by Agew-Awi and Amhara people in northwest Ethiopia.[5] In other ethnobotanical studies, *C. myxa (*seeds), *C. rothii* (fruit pulp), *C. americana* (stem), and *C. retusa* (leaves), all belonging to family Boraginaceae as *C. africana*, are reported to be used in the traditional treatment of diarrhea.[6–9] Moreover, there are in vitro tests on antibacterial activity and other essential biological activities (antioxidant, anti-inflammatory, cytotoxic) and phytochemical screening test reports (on *C. africana* and plants of the same family), which can be considered as starting evidences to test the antidiarrheal activities of *C. africana* in animals.[12–14,25–28] There are also other current reports on plant-based studies showing the antidiarrheal effects of plant extracts in experimental animals.[29–31] Such evidences encourage to undertake laboratory-based studies to confirm the claimed traditional use of plants scientifically.

The results of this study showed that the extract of *C. africana* produced a statistically significant reduction in castor oil-induced diarrhea and intestinal fluid accumulation in a dose-dependent manner, especially the effect of the maximum dose being closer to the effect of loperamide. Also, the extract significantly reduced intestinal transit to similar level to that of atropine as revealed by decrease in the portion of intestinal length traversed by charcoal meal.

Although the extract was found to reduce castor oil-induced diarrheal episodes, enteropooling, and intestinal motility, mechanism of its activity is uncertain. Diarrheal effect of castor oil is mediated by several mechanisms. Castor oil is hydrolyzed in the small bowel by lipases to glycerol and the active agent ricinoleic acid, which is poorly absorbed from intestinal lumen. This active agent acts primarily in the small intestine to stimulate secretion of fluid and electrolytes, decrease intestinal absorption of water and electrolytes and speed intestinal transit due to peristalsis that is increased secondarily.[32,33] The antidiarrheal effect of the plant extract can be proposed from the mechanism of action of castor oil that leads to induction of diarrhea. The antidiarrheal activity of the plant could be mediated by counter activity to the mechanisms that mediate diarrhea induction by castor oil, ie, the extract may act to increase water and electrolyte absorption or decrease the secretion of fluid and electrolytes. This may be the mechanism that enables the extract to decrease the intestinal fluid overload and the diarrheal episodes in a dose-dependent manner. The mechanism of antidiarrheal action of the plant extract also may be via the inhibition of the effect of the ricinoleic acid on prostaglandin E_2 receptors. Blocking the prostaglandin receptors can decrease or prevent secretary and intestinal motility effect of castor oil and prostaglandins that can be released due to intestinal irritating effect of ricinoleic acid. On the other hand, the extract may act by abolishing peristalsis in the colon and hence significantly delaying passage of feces through the bowel. The delayed transit through the bowel also increases the opportunity for increased absorption of fluid from the feces, producing a drying effect on the stool that further slows its progress through the bowel. This is consistent with the mechanism of action of loperamide for its antidiarrheal effect.[34]

Table 4 Effect of methanolic extract of *C. africana* on intestinal transit in mice

Groups	Dose (mg/kg)	Traversed by charcoal meal (%)	Inhibition intestinal length traversed by charcoal meal (%)#
Group 1 (water), negative control	–	79.02±4.85	0
Group 2 (atropine), positive control	1	26.48±2.68^{a***}	66.49
Group 3 (extract)	100	59.73±3.10^{a*,b***}	24.41
Group 4 (extract)	200	47.50±5.96^{a***,b***,c**}	39.89
Group 5 (extract)	400	38.20±2.34^{a***,b**,c***,d*}	51.66

Notes: Values are in mean ± SEM (n=6). [a]Compared with negative control; [b]compared with positive control; [c]compared with group 3; [d]compared with group 4. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001. #Inhibition (%) is relative to the negative control group.
Abbreviations: *C. africana*, *Cordia africana*; SEM, standard error of mean.

The antisecretory and hence antidiarrheal effect of the extract may also be attributed to the probable counter activity to the generation or activity of cyclic nucleotides, cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate. An increase in either cAMP or cyclic guanosine monophosphate has been shown to stimulate chloride ion (Cl^-) secretion and simultaneously inhibit Na^+/Cl^- absorption. Various endogenous hormonal regulators of ion secretion and absorption, such as prostaglandins and vasoactive intestinal polypeptide, have been demonstrated to trigger Cl^- secretion by generating increased levels of cAMP.[34] The antimotility effect of the plant extract may be by mechanisms that involve the inhibition of cholinergic activity and serotonergic activity on the small intestine. Serotonin is released into the blood postprandially and in response to changes in pressure across the gut wall as well as to noxious stimuli, and it has been reported that the activation of serotonergic receptors, 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors, by endogenous serotonin cause gut motility and affects bowl transit in experimental animals.[34,35] So, antiserotonin action can be considered as the mechanism of action by which the plant extract reduced intestinal motility significantly. Atropine is an anticholinergic and will therefore reduce gastrointestinal motility.[36] It is reported that 5-HT-induced contraction of circular muscles is blocked completely by atropine and hexamethonium in animals, indicating that 5-HT stimulates intestinal contractions through the release of ACh.[35] Like atropine, the extract may inhibit excitation of myenteric and mucosa plexuses and cause reduction in intestinal motility and secretion.

The phytochemical analysis of methanolic root bark extract of *C. africana* revealed the presence of phenols, flavonoids, terpenoids, and saponins in line with the report from Sudan.[11] These secondary metabolites are reported to have antidiarrheal and antimicrobial activities. The mechanism of action of these chemical components for their antidiarrheal effect is also reported; saponins inhibit histamine release in vitro; terpenoids inhibit release of autacoids and prostaglandins; phenols make intestinal mucosa more resistant, reduce secretion and the intestinal transit and have astringent action; and flavonoids inhibit release of autacoids and prostaglandins. Together with this, their respective mechanism of antimicrobial activity is also reported.[12] These chemical constituents are probably responsible for the antidiarrheal (as confirmed in this study) and antibacterial effect (report from previous study) of the plant extract.

As demonstrated in the result section, since the extract does have promising antidiarrheal effect, the plant may be useful in the management of a wide range of diarrheal states due to disorders of transit or due to abnormal secretory mechanisms like in cholera or *E. coli* enterotoxin-induced diarrhea, especially in compelling conditions where modern drugs like loperamide and other antidiarrheal drugs and antibiotics are not available.

Conclusion

To iterate the findings, the root bark extract of *C. africana* was found to be effective in preventing castor oil-induced diarrhea and intestinal motility significantly, especially at the maximum dose. The secondary metabolites identified may be responsible for the antidiarrheal activity of the plant through various possible mechanisms of action proposed.

Acknowledgment

The authors would like to acknowledge University of Gondar, Research and Community Service Core Process for giving financial support to undertake this study.

Author contributions

All the authors participated in this work from proposing the title and proposal development to writing up of the final report. The manuscript preparation to be submitted for publication was prepared by ABA and EMB. The laboratory activities, data compilation and analysis, and draft report preparation were done by ABA. Moreover, all the authors are equally responsible for any issue related with the appropriateness of this work.

Disclosure

The authors report no conflicts of interest in this work.

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