Evaluation of the antidiarrheal activity of the leaf extract of *Parquetina nigrescens* and formulation into oral suspensions

**Purpose:** *Parquetina nigrescens* (Pn) extract was evaluated for safety and antidiarrheal activity, formulated into stable suspensions, and characterized.

**Methods:** Acute toxicity of the extract based on Organization for Economic Cooperation and Development-423 guidelines was performed. The antidiarrheal effects of the extract on castor oil-induced diarrhea in four groups of Wistar rats were determined. The first and second groups received 5 and 200 mg/kg body weight (bw) of the extract, while the third and fourth groups received normal saline (5 mg/kg bw) and loperamide (5 mg/kg bw) as negative and positive controls, respectively. Pn extract was used at 1.25% w/v to formulate structured vehicle (carboxymethylcellulose, polyvinylpyrrolidone and tragacanth) suspensions. The suspensions were tested for pharmacological activity and characterized.

**Results:** Acute toxicity gave a lethal dose 50 (LD50) that is greater than 300 and less than 2000 mg/kg bw. A reduction in intestinal transit by 0.14 and 0.15% at 5 and 200 mg/kg of the extract was achieved as compared to an inhibition of 0.12% by 5 mg/kg loperamide. There was a dose-dependent decrease in the frequency of watery stool passed in castor oil-induced rats by 35.29% and 64.70% at 5 and 200 mg/kg, respectively. All the suspensions inhibited diarrhea, exhibiting a dose-dependent pattern and remained stable after 4 weeks. Their pH values ranged from 4.60 ±2.73 to 4.73±1.91, while viscosity ranged from 3.50±1.23 to 6.75±1.24 Pas at 60 rpm.

**Conclusion:** The results suggest that Pn possesses significant antidiarrheal activity. Suspensions of Pn were successfully formulated in structured vehicles and were effective in the control of diarrhea in Wistar rats.

**Keywords:** *Parquetina nigrescens*, acute toxicity, structured vehicle, antidiarrhea

**Introduction**

In developing countries, such as Nigeria, diarrhea is one of the leading causes of morbidity and mortality in both children and adults. This is mainly due to poor hygiene and sanitation. Diarrhea kills more children than malaria, measles and AIDS combined.1 According to WHO, diarrhea is defined as the passage of three or more loose or liquid stools per day or more frequent passage than is normal for the individual. The most common cause of diarrhea is disruption of the epithelium of the intestine by various types of bacteria, virus and parasites. Although in many clinical cases, the actual cause of the disease is unknown. The incidence of diarrhea remains high, despite the efforts of international organizations to control the disease.2 In diarrhea, there is a change in bowel movement that results in an increase in water content and thus an increase in the frequency of stools.2
infection can be spread out through infected food, contaminated drinking water and unhygienic environment. Aside interventions such as ensuring proper hygiene, improved sanitation and rehydration, several orthodox medicines have been used to treat diarrhea. Due to the increasing side effects and development of resistance to orthodox medicine, new and safe drug sources are being discovered from plant origin. There has been a tremendous surge in the acceptance of natural therapies as an alternative form of health care in both developing and developed countries due to their affordability, ease of accessibility and safety. From thousands of years ago, plants have provided a reservoir of compounds used as therapeutic agents. Active metabolites such as alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols found in plants are responsible for their medicinal properties. In the world today, plants are being used to treat several health concerns and conditions. Medicinal plants with anti-diarrheal properties have been widely used by the traditional healers. However, the effectiveness of many of these plants has not been scientifically evaluated and proven. One of such plant is Parquetina nigrescens (Pn).

Pn belongs to the family Apocynaceae. Its synonym is Periploca nigrescens. Pn occurs widely in Africa: from Senegal east to Sudan and south through Central and East Africa to Zambia, Angola and Eastern Zimbabwe. From folklore, a leaf decoction or infusion of Pn is drunk to treat diarrhea and other diseases. A leaf decoction with honey added is also drunk to treat fatigue, jaundice, stomach ulcers and anemia, as a tonic. It is also taken to treat hypotension and to ease childbirth. The body is washed with a leaf decoction to treat general fatigue. The leaf is a common ingredient in medications used to treat insanity. Based on these claims, this investigation attempts to determine the acute toxicity of Pn extract in rats; to evaluate antidiarrheal effects of this plant via the determination of the effect of the aqueous leaf extract of Pn on castor oil-induced diarrhea in rats and determination of the effect of the extract on gastrointestinal tract motility in rats. If this pharmacological activity of interest is validated, suspensions can be made of the extract as an alternative in improved management of diarrhea, especially in children.

Pharmaceutical products are formulated by combining active pharmaceutical ingredient (API) with different excipients based on the dosage form intended, to produce a medicinal product. They are formulated into tablets, medicinal gases, solutions, suspensions, ointments, creams and gels in order to facilitate the delivery of APIs to the patient’s system. Liquid formulations such as suspensions have their APIs combined in liquid vehicles; this poses a challenge of poor aqueous solubility which may affect the bioavailability of active ingredients. A suspension is a two-phased system with uniform dispersion of finely divided solid drug particles in a continuous phase of solid, liquid or gas in which the drug has minimum solubility. The suspension has long been used to improve the bioavailability of poorly soluble active ingredient, mask the bitter taste, and improve stability of active ingredients. Suspensions are mostly unstable and often tend to sediment overtime if left without agitation. Physical, chemical and microbiological stability can be achieved by the addition of appropriate excipients. A good suspension should have good odor, color and taste and low sedimentation rate; should be physically and chemically stable; should be easy to re-disperse with minimal agitation and should resist microbial growth. The choice of suspension in this research was to widen the range of usability, improve the stability of the herbal formulation and offer a faster therapeutic response.

Materials and methods

Material

Fresh leaves of Pn, milling machine, methanol, electronic balance, rotary evaporator, filter paper, water bath, distilled water, viscometer and pH meter. DMSO, normal saline, castor oil, Wistar albino rats, gelatin, filter paper, loperamide, pharmaceutical grades of tragacanth, carboxyl methyl cellulose (CMC), polyvinyl pyrrolidone (PVP), polysorbate 80, sodium chloride and glycerin were obtained from a chemical laboratory.

Plant collection

Fresh leaves of Pn were collected between January and February 2017 from Ilorin, Kwara State, Nigeria. The plant was identified and authenticated at the Department of Plant Biology Faculty of Life Sciences, University of Ilorin Herbarium, and assigned the voucher number UILH/067/876. The leaves were air-dried for 2 weeks.

Plant extraction

The dried plant was milled using a milling machine, and aqueous methanol (1:3) as solvent was used to macerate the milled plant. The extract was evaporated using a rotary evaporator and subsequently freeze-dried. The product obtained was stored at 4°C.
Ethical approval
The Institutional Ethical Committee of University of Ilorin, Ilorin Nigeria, approved the protocol for this study under the number UERC/ASN/2017/914.

Acute toxicity study
According to the OECD-423 guidelines, Wistar albino rats were selected by random sampling. The animals were kept fasting overnight and provided with water only. 2,000 mg/kg body weight of the extract was administered orally by gastric intubations, and the rats were observed for 14 days. Mortality was observed in three out of the three animals; therefore, the dose administered was assigned toxic dose. The concentration of the extract administered was stepped down to 300 mg/kg body weight, and the rats were observed for 14 days. No mortality was observed. Therefore, the same dose was repeated to confirm the safe dose of the extract.

Test for antidiarrheal activity
Animals
Wistar albino rats of either sex (90–100 g) were collected and acclimatized to normal laboratory conditions for 1 week prior to study and given pellet diet and water ad libitum. Ethical approval was obtained, and the animals were handled according to the guideline of National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85–23 revised 1985).

Castor oil-induced diarrhea in rats
Rats of either sex were divided into four groups of five rats each. The animals were fasted for 18 hrs prior to the test. Group I rats were treated with normal saline (2 mL/kg), which served as control, while Group II received loperamide (5 mg/kg). Group III received 5 mg/kg extracts of Pn and Group IV received 200 mg/kg extracts of Pn. All doses were administered orally. After 1 hr, all groups received 1 mL of castor oil orally. The animals were placed in cages lined with adsorbent papers and observed for 4 hrs for the presence of diarrhea defined as watery (wet), unformed stool. The control group result was considered as 100%. The activity of each group was expressed as percent inhibition (%) of diarrhea. The percentage inhibition of defecation was calculated as follows:

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\text{% Inhibition of defecation} = \frac{A - B}{A} \times 100
\]

where \( A \) indicates the mean number of defecations caused by castor oil and \( B \) indicates the mean number of defecations caused by drug extract.

Gastrointestinal motility test
For the test, selected rats were divided into four groups of five rats in each. At first, 1 mL of castor oil was given orally to every rat of each group to produce diarrhea. After 1 hr, Group I (control group) received saline (2 mL/kg) orally. Group II received standard drug (loperamide 5 mg/kg) and Groups III and IV received 5 mg/kg and 200 mg/kg extract of Pn respectively. After 1 hr, all animals received 1 mL of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, following the charcoal meal administration, all animals were euthanized, and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, was measured and expressed as percentage of the total length of the small intestine from pylorus to the caecum.

Intestinal transit % = \( \frac{\text{Length travelled by black marker}}{\text{Total length of small intestine}} \times 100 \)

Percentage of transit inhibition = \( \frac{(T_0 - T_1)}{T_1} \times 100 \)

where \( T_0 \) = intestinal transit of saline (control) and \( T_1 \) = intestinal transit of extract.

Preparation of Pn suspension
This was carried out according to the method of Eskanda et al with slight modification. Appropriate quantities of CMC, PVP and tragacanth were weighed as presented in Table 1, sieved with a size 120-mesh sieve and validated according to a standard procedure. A paste of the structured vehicle (CMC, PVP and tragacanth) base was made with the ingredient combination in Table 1 for formulations A–D and allowed to stand for 10 mins. 1.25% w/v of freeze-dried extracts of Pn was weighed and dispersed into a mixture of the wetting agents, polysorbate 80 and glycerol. The paste was scooped into the extract solution in bits, and the resultant system was mixed systematically for a sufficient duration of time. The flocculating agent, NaCl, was added while the system was being mixed. The mixing was continued until a uniform and consistent suspension was obtained. The formulation was tested for pharmacological action. Three different formulations (A, B and C) were made by varying the composition of the structured vehicle according to Table 1. Formulation D prepared without the extract served as the negative control.
Pharmacological action of suspensions of methanol extract of Pn

Rats of either sex were divided into four groups of three rats each. The animals were fasted for 18 hrs prior to the test. Group I received 5 mg/kg suspension of Pn, Group II received 200 mg/kg suspension of Pn, Group III received loperamide (5 mg/kg), while Group IV rats were treated with normal saline (2 mL/kg), which served as control. All doses were administered orally. After 1 hr, all groups received 1 mL of castor oil orally. The animals were placed in cages lined with absorbent papers and observed for 4 hrs for the presence of diarrhea defined as watery (wet), unformed stool.

Physical enhancement of suspensions of methanol extract of Pn

On confirmation of pharmacological action, the formulations were enhanced with coloring and flavoring agents in sufficient amounts. The resultant suspensions were dispensed into clear glass bottles and stored for analysis.

Analysis of organoleptic properties and pH of suspensions of methanol extract of Pn

The physical properties of the suspensions such as color, odor, appearance, sedimentation and pourability were observed by direct perception and compared to a standard suspension. The suspensions were also observed physically for the presence of sediments over a period of 30 days. The pH value of the formulations was determined at room temperature using a pH meter (Hanna Instruments Limited, Leighton Buzzard, UK) calibrated to pH 7. The analysis was performed in triplicates.

Evaluation of viscosity of suspensions of methanol extract of Pn

The viscosities of the formulations were determined using an NDJ-5S Digital Display Viscometer (Shanghai Geological Instrument Co. Ltd, Shanghai, China) at 25±1°C. Spindle size 4 was used at speed 6, 12, 30 and 60 rpm.

Stability studies of suspensions of methanol extract of Pn

The formulations were left in clear bottles for 30 days at 25°C (room temperature) and 40°C (accelerated temperature).

Statistical analysis

The results were expressed as the mean ± SEM. Data obtained were analyzed using graph pad prism. Statistical analysis was carried out using one-way ANOVA. Statistical significance was taken at \( p<0.05 \).

Results

The oral acute toxicity of the extract LD\(_{50}\) was found to be 300 mg/kg body weight > LD\(_{50}\)<2000 mg/kg body weight. The toxicity indices for agility, tremor, convulsion, breathing pattern, lethargy and coma exhibited by the animal groups are as shown in Table 2.

The number of watery stools passed in castor oil-induced rats and % inhibition of 5 and 200 mg/kg as compared with Loperamide\(^\text{®}\) and castor oil-induced (control) groups are presented in Table 3.

The intestinal transit of charcoal meal was measured and are presented in Table 4. The physical properties (color, odor, appearance, sedimentation and pourability) of suspensions of methanol extract of Pn are presented in Table 5.

In Table 6, the pH of the suspensions ranged from 4.60±2.73 to 4.73±1.91. Viscosity at 6 to 60 revolutions per minute (rpm) was also measured for the suspensions.

Pharmacological actions of the suspensions of methanol extract of Pn demonstrated by the number and amount of stool passed by the test animals are presented in Table 7.

While the onset of pharmacological action due to administered suspension of methanol extract of Pn on the test animal groups is presented in Table 8.

Discussion

Herbal medicine as an alternative and complementary therapy is now gaining grounds in both developing and
advanced countries. From literature, Pn has shown potential for the treatment of a variety of diseases. One such disease is diarrhea, which is one of the leading causes of death in both children and adults in Africa. Herbal medicines are commonly used today due to their limited side effects as compared to conventional medicines.

From the result of the study, a single-dose oral administration of the extract at 2,000 mg/kg body weight caused mortality in the rats. A concentration of 300 mg/kg body weight had no effect on mortality in the rats. According to Global Harmonized System of Classification and Labelling of Chemicals (GHS), the extract is classified in category 4 defined by oral LD$_{50}$ in the range of 300 to 2,000 mg/kg body weight and the toxicity indices are presented in Table 2. In Table 3, there was a dose-dependent decrease in the number of watery stools passed in castor oil-induced rats by 35.29% and 64.70% at 5 and 200 mg/kg, respectively. However, at equivalent concentration of the
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### Table 2: Toxicity index exhibited by the animal groups

| Animal group | Toxicity index (+) mild, (+++) moderate, (++++) severe, (–) nil |
|--------------|-----------------------------------------------------------------
| First Dose (2,000 mg/kg) | Agility | Tremor | Convulsion | Breathing pattern | Lethargy | Coma |
| Animal 1 | ++ | + | – | – | ++ | +++ |
| Animal 2 | +++ | – | – | – | ++ | +++ |
| Animal 3 | ++ | + | – | – | ++ | +++ |

| Second Dose (300 mg/kg) | Agility | Tremor | Convulsion | Breathing pattern | Lethargy | Coma |
| Animal 1 | + | – | – | – | – | – |
| Animal 2 | ++ | – | – | – | – | – |
| Animal 3 | – | – | – | – | – | – |
| Animal 4 | ++ | – | – | – | – | – |
| Animal 5 | ++ | – | – | – | – | – |

### Table 3: Effects of methanol extract of the leaves of *Parquetina nigrescens* (Pn) on castor oil-induced diarrhea in rats

| Treatment | Dose (mg/kg) | Number of watery diarrheas | % inhibition |
|-----------|-------------|-----------------------------|--------------|
| Castor oil (control) | 1.00 mL | 17.00±1.29 | – |
| Pn extract | 5.00 | 11.00±0.81 | 35.29 |
| Pn extract | 200.00 | 6.00±0.39 | 64.70 |
| Loperamide | 5.00 | 8.00±0.54 | 52.94 |

Note: $p<0.05$.

### Table 4: Effects of methanol extract of the leaves of *Parquetina nigrescens* (Pn) on gastrointestinal transit in rats

| Treatment | Dose (mg/kg) | Mean intestinal length (cm) | Mean distance travelled by charcoal (cm) | Mean intestinal transit % inhibition |
|-----------|-------------|-----------------------------|----------------------------------------|-----------------------------------|
| Saline (control) | 2.00 mL | 99.23±2.56 | 37.43±3.82 | 0.38±0.82 | – |
| Pn extract | 5.00 | 98.34±1.32 | 23.37±1.27 | 0.24±0.24 | 0.14 |
| Pn extract | 200.00 | 96.67±1.08 | 22.66±1.38 | 0.23±0.17 | 0.15 |
| Loperamide | 5.00 | 91.23±1.29 | 23.49±1.22 | 0.26±0.33 | 0.12 |

Note: $p<0.05$.

### Table 5: Physical properties of suspensions of methanol extract of *Parquetina nigrescens*

| Properties | Formulation A | Formulation B | Formulation C | Formulation D | Plain Formulation without extract |
|------------|---------------|---------------|---------------|---------------|----------------------------------|
| Color      | Sangria       | Sangria       | Sangria       | Crimson       | White                            |
| Odor       | Pleasant      | Pleasant      | Pleasant      | Pleasant      | Pleasant                         |
| Appearance | Glossy, thick | Thick         | Thick         | Clear, thick  | Thick                            |
| Sedimentation | No sediments | No sediments | No sediments | No sediments | No sediments                     |
| Pourability | Not easy      | Easy          | Easy          | Easy          | Easy                             |
extract and loperamide, 5 mg/kg body weight, loperamide appeared to produce a greater inhibition. This may be attributed to the long duration of action of loperamide. The results obtained showed that the extract of Pn has a great potential to reduce the frequency of stool at a dose comparable to that of the conventional drug used as control.

In the presence of castor oil in the gut, prostaglandins are secreted, which in turn induces gastrointestinal motility. Hypermotility is one of the different pathophysiological conditions that characterize diarrhea. The charcoal meal test was carried out to determine the effect of Pn extract on gut motility. Loperamide was used as a control as it has been known to inhibit castor oil-induced diarrhea. As shown in Table 4, it was observed that Pn extract reduced the intestinal transit of charcoal meal by 0.14% and 0.15% by 5 and 200 mg/kg of the extract, respectively, as compared to an inhibition of 0.12% by 5 mg/kg loperamide. A reduction in intestinal transit time shows that the extract was able to reduce the motility of the gut and thus reduce diarrhea. Normal saline, which was the negative control, showed no inhibition, as it will normally not have effects on diarrhea. The result also showed that at 5 mg/kg body weight of extract and loperamide, the levels of inhibition produced by the extract were greater than that produced by loperamide. It can, therefore, be said that only a small concentration of extract is required for antidiarrheal effect and thus less amount of drug in the system. This also implies less toxic effect. Having established the antidiarrheal effect of Pn, it is pertinent to formulate the extract into dosage forms that can ease administration, mask the characteristic odor and color of the plant and elicit a faster onset of action, hence, the choice of a suspension.

This research was carried out to formulate the extract of Pn into a suspension, validate its antidiarrheal effect as a suspension for oral administration and characterize the suspension. All the suspensions inhibited diarrhea in a dose-dependent pattern. As the concentration of extract increased, there was a remarkable decrease in the number of loose stools passed by the test animals. A relatively good level of compatibility was observed between the

### Table 6 pH and viscosity of suspensions of methanol extract of *Parquetina nigrescens*

| Formulation ID | pH         | Viscosity (Pas) |
|---------------|------------|-----------------|
|               |            | 60 rpm          | 30 rpm          | 12 rpm          | 6 rpm          |
| A             | 4.60±2.73  | 6.21±0.18       | 3.80±2.29       | 2.54±1.04       | 4.90±4.22      |
| B             | 4.64±1.64  | 5.70±3.22       | 3.40±0.23       | 1.80±3.33       | 4.40±2.67      |
| C             | 4.73±1.91  | 3.50±1.23       | 1.80±1.19       | 3.00±2.23       | 1.54±0.35      |
| D             | 6.50±0.23  | 6.75±1.24       | 4.14±0.01       | 2.35±3.58       | 8.50±1.92      |

Note: p<0.05.

### Table 7 Pharmacological action of the suspensions of methanol extract of *Parquetina nigrescens*

| Test animals | Group I (suspension 5 mg/kg) | Group II (suspension 200 mg/kg) | Group III (positive control) | Group IV (negative control) |
|--------------|------------------------------|---------------------------------|------------------------------|------------------------------|
| Rat 1        | 2 loose stools               | 1 loose stool                   | 3 loose stools               | 4 loose stools               |
| Rat 2        | 3 loose stools               | 2 loose stools                  | 3 loose stools               | 4 loose stools               |
| Rat 3        | 2 loose stools               | 1 loose stool                   | 4 loose stools               | 4 loose stools               |

Note: Positive control = loperamide suspension.

### Table 8 Onset of pharmacological action due to administered suspension of methanol extract of *Parquetina nigrescens*

| Test groups | 0–15 mins | 15–30 mins | 30 mins–1 hr | 1–2 hrs | 2–3 hrs | 3–4 hrs |
|-------------|-----------|------------|--------------|---------|---------|---------|
| Group 1     | *         | *          | *            | *       | *       | *       |
| Group 2     | *         | *          | *            | *       | *       | *       |
| Group 3     |           |            |              |         |         |         |
| Group 4     |           |            |              |         |         |         |

Notes: *Passage of stool; Group 1 – animals administered 200 mg/kg suspension; Group 2 – animals administered 5 mg/kg suspension; Group 3 – negative control; Group 4 – positive control (loperamide suspension 5 mg/kg).
ingredients of the suspension and plant extract as there was only a slight decrease in the pharmacological activity of the suspension as compared to the extract only. The choice of a structured vehicle was to improve the stability of the product and aid easy re-dispersion at the time of administration. In a structured vehicle, the viscosity of the preparation approaches infinity on storage under conditions of very low shear. The vehicle can maintain the suspended particles, thereby conferring stability.

All the suspensions were appealing to sight, smelt good and showed no visible sediments as presented in Table 5. Formulation A was very thick and did not flow easily from the container, while the other formulations flowed easily. The pH of the suspensions ranged from 4.60±2.73 to 4.73±1.91 as presented in Table 6. The pH value of an aqueous solution represents its degree of acidity or alkalinity, and the pH of a preparation must be optimum as they are administered. The slightly acidic pH values obtained are optimal for formulations intended for oral administration.21 The viscosity of the suspensions was dependent on the components of structured vehicle used. Formulation A containing all three components of the structured vehicle had the highest viscosity as shown in Table 6. However, the control suspension gave viscosity values higher than the test suspensions. This implies that the presence of plant extracts lowered the viscosity of the formulations. At the end of the stability studies, the suspension showed a slight change in pH considered statistically insignificant. The color and odor remained unchanged with no visible sediments. This result shows that the particles of the extract will continue to remain suspended in the vehicle, thereby remaining stable.

Pharmacological analysis was carried out which validated the antidiarrheal properties of Pn suspension when administered orally. This was in line with the pharmacological effects obtained when the extract was analyzed. There was a dose-dependent decrease in the number and amount of stool passed by the test animals as presented in Table 7. The effect obtained at a concentration of 5 mg/kg body weight of formulated suspension was comparable to that of the control drug used, with 200 mg/kg having a greater effect. This implies that optimal diarrhea control can be achieved between the doses of 5 and 200 mg/kg. From Table 8, 200 mg/kg concentration of the formulated suspension produced diarrhea in the test group between 2 and 3 hrs while the group treated with 5 mg/kg produced diarrhea between 1 and 2 hrs. An equivalent concentration of the control drug produced diarrhea in the test group between 30 mins to an hour. Therefore, it can be said that in addition to inhibiting the passage of loose stools in test animals, suspensions of Pn were able to maintain the inhibition for up to an hour, thereby confirming a longer duration of pharmacological effect and thus reducing the need for repeated dosing. All numerical data obtained are reported as averages. An ANOVA for the different mean results gave “p-values” <0.05 at every instance, implying that each average is statistically significant and different from the other on comparison.

Conclusion
The results of the study suggest that methanol extract of the leaves of Pn possesses antidiarrheal properties. Suspensions of Pn were successfully formulated in structured vehicles of a combination of CMC, PVP or tragacanth. The suspension exhibited antidiarrheal activity by inhibiting gastric motility in mice. Further investigations will be carried out to validate the dose of the plant extract that produced the best activity in formulation and develop the drug moiety into dosage forms that can be subjected to clinical evaluation.

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Disclosure
The authors report no conflicts of interest in this work.

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