EVALUATION OF ANTI-DIARRHEAL ACTIVITY OF ETHANOLIC LEAF EXTRACT OF ERIOBOTRYA JAPONICA LINDL

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ABSTRACT

Objective: The study was carried out to investigate the antidiarrheal activity of ethanolic leaf extract of Eriobotrya japonica (EEJ) using various models of experimental diarrhea.

Methods: Antidiarrheal property of EEJ at 100, 200, and 400 mg/kg/bwt was evaluated using castor oil-induced diarrhea, castor oil-induced enteropooling, and gastrointestinal propulsive models of experimental diarrhea in Sprague Dawley rats of both sexes, weighing 200–250 g. Cytotoxicity test of EEJ was performed using brine shrimp bioassay.

Results: Toxicity assay of EEJ showed a lethal concentration value of 1225 µg/ml suggesting non-toxicity. EEJ significantly (p<0.05) and dose-dependently (100, 200, and 400 mg/kg/bwt) inhibited castor oil-induced diarrhea by 38.1%, 76.19%, and 100%, respectively, and enteropooling by 28%, 56%, and 88%, respectively, compared with control. Pre-treatment with yohimbine, α2-adrenoceptor blocker significantly reversed the protective effect of EEJ (400 mg/kg) against castor oil-induced diarrhea and against castor oil-induced enteropooling, suggesting the involvement of α2-adrenoceptors in antidiarrheal property of EEJ. Furthermore, EEJ significantly (p<0.05) and dose-dependently (100, 200, and 400 mg/kg/bwt) inhibited gastrointestinal motility by 28%, 62%, and 89.2%, respectively.

Conclusion: The study has demonstrated the antidiarrheal potential of ethanolic leaf extract of EEJ, which may be attributable to its dual antisecretory and antimotility activities probably through activation of the sympathetic α2-adrenergic pathway.

Keywords: Eriobotrya japonica, Enteropooling, α2-adrenoceptors, Cytotoxicity, Anti-secretory.

INTRODUCTION

Diarrhea is a common gastrointestinal disorder affecting people of all ages worldwide. Diarrhea is usually characterized by the passage of a large volume of loose or watery stool at an increased frequency usually 3–4 times a day or stool volume >200 ml/day irrespective of the cause, be it infectious or not [1-3]. Diarrhea results from an imbalance between absorptive and secretory processes that occur in the intestinal mucosa, and it is associated with excessive loss of essential electrolytes such as sodium ions, potassium ions, and chloride ions leading to dehydration and electrolyte imbalances [4,5]. Diarrheal diseases are usually associated with low living standards, poor sanitation infrastructure and poor access to portable, and safe-treated water sources [6,7]. Many rural dwellers depend mostly on the pond and river waters, whereby the community members share with household animals [8,9]. Reports suggest that about 2.4 billion people do not have basic sanitation with 1.1 billion people lacking access to improved and treated water supply worldwide [10]. South Africa is commended for achieving the water and sanitation targets for the millennium development goal with over 90% of South Africans having access to clean public water. However, estimates indicate that there are 6 million households and 1.4 million households which still lack access to clean water and sanitation services, respectively [11], putting this group of people at risk of getting diarrheal diseases.

Diarrhea leads to death if dehydration and electrolyte loss persist and become too severe without rehydration or treatment [12,13]. Worldwide, diarrhea claims several million lives annually and is reportedly to be the second leading cause of deaths mostly those of infants [14]. For instance, global mortality estimate from diarrhea is reported to be over 1.5 million deaths of children under the age of five annually [15]. In 1998, a estimated number of people killed by diarrhea worldwide were 7.1 [16]. The prevalence and incidences of diarrhea are on the rise especially in developing nations including South Africa. For instance, every year, South Africa reports about 3 million incidents of diarrheal cases requiring treatment. Furthermore, in South Africa alone, every year, and diarrheal diseases are claimed to be responsible for about 43000 deaths, with under five deaths accounting for 20% [17].

Diarrhea is treated with a number of available conventional drugs the most efficacious of which is loperamide, which acts both as antisecretory and antimotility drug in the intestines [18]. However, use of these conventional drugs has some limitations as some present with undesirable effects such as vomiting, fever, constipation, bronchospasms, dry mouth, and abdominal pains among others [19] and in addition, most infectious diarrhea are treated with a course of antibiotics, which currently is faced with massive drug-resistant challenges [20,21]. Furthermore, they are expensive and not easily accessible to ordinary poor rural communities. In most cases, they are not even available due to lack of adequate health facilities such as clinics and hospitals that are located at a very far distance from the community members [22]. It is because of these limitations that people resort to use of herbal remedies and other alternative treatments which have been perceived to be effective, affordable, and accessible and indeed with fewer or no side effects.

Worldwide, about 80% of communities rely on herbal remedies to treat various human ailments including diarrhea and other gastrointestinal
disorders [23]. Moreover, the WHO has encouraged the use of traditional medicinal plants for the treatment and prevention of diarrhea worldwide [24, 25]. A number of medicinal plants have been evaluated for their antidiarrheal properties, and reports indicate that they possess antispasmodic activities, delay gastrointestinal transit, inhibit motility of the gut, and reduce the intraluminal fluid accumulation with a concomitant enhancement of water and ion absorption [26, 27]. Eriocheir japonicus (EEJ) Lindl. commonly known as locust tree is one of such plants which is popularly and commonly used in the traditional medicine practice by several communities in treating various human diseases including diarrhea and other gastrointestinal-associated disorders by many rural communities in South Africa, especially in the Eastern Cape Province. Several biological activities of this plant have been reported. For instance, extracts obtained from this plant are reported to possess anti-inflammatory, antidiabetic, antioxidant, antiallergy, hyperleptinemia, hyperinsulinaemia, and several other disorders [28-32]. Despite its long traditional use in the treatment of diarrhea, there is little documented scientific information regarding its antidiarrheal properties. The present study, was, therefore, conducted to scientifically evaluate the antidiarrheal effect of ethanolic leaf extract of EEJ using various models of diarrhea in an attempt to establish its efficacy and possible mechanism(s) of actions.

**METHODS**

**Plant material**

Leafy parts of EEJ were collected from areas surrounding Walter Sisulu University, Mthatha Campus. Identification and authentication of the plant were done by a senior taxonomist of the herbarium section, Department of Biological Sciences Walter Sisulu University, Mthatha Campus. A herbarium specimen was prepared and deposited with a voucher number: Nomathithi 1 (KEI).

**Preparation of ethanolic leaf extract of EEJ**

The leaves were shredded and allowed to air-dry under shade at room temperature for 3 weeks. The dried leaves were then milled into powder using a commercial blender. The powdered material was soaked in 70% ethanol and allowed to stay for 2 days with continuous shaking using (Labcon) platform shaker machine at room temperature. The resultant mixture was filtered using Whatman No. 1 filter paper in Buchner funnel. The filtrate was concentrated using the rotary evaporator under reduced pressure at 55°C. The semi-solid concentrated extract was finally dried in an oven at 40°C to obtain a crude extract which was designated EEJ and was kept in a bio-freezer at −75°C until the day of experiments.

**Animals**

Sprague Dawley rats of both sexes, weighing 200–250 g were used for the study. Rats were supplied by Shalom Laboratories and housed in the storage facility in the Department of Physiology, Walter Sisulu University. The animals were kept in polypropylene open top cages (four in each cage) and were maintained at an ambient temperature of 25 ± 2°C and 12 h light/12 h dark regime. The animals were acclimatized to the animal housing storage unit and laboratory conditions for 2 weeks before the commencement of the experiment and during this period, animals were fed with standard rat pellet chow (Epol SA) and water ad libitum. The experimental protocol and all procedures were approved by the Faculty of Health Sciences Research Ethics Committee, Walter Sisulu University. Animals were handled in compliance with the Guide for care and use of Laboratory Animals (NIP Publication No. 85 (23), revised in 1996).

**Experimental design**

The cytotoxicity test of the extract was performed using Brine Shrimp lethality bioassay. To establish the antidiarrheal activity of EEJ, the extract was screened against various models of diarrhea, namely castor oil-induced diarrhea, castor oil-induced enteropooling, and castor oil-induced gastrointestinal propulsion in rats. Before commencement of the experiments, the rats were fasted either for 18 or 24 h depending on the experimental procedure. Administration of the extract and loperamide was performed by intragastric gavage using straight feeding needles suitable for the animal depending on its size, whereas yohimbine and atropine were administered intraperitoneally (ip) in live animals.

**Determination of cytotoxicity of EEJ**

The cytotoxic effect of EEJ was carried out using Brine shrimp lethality bioassay according to the model described by Nguta et al. [33], with slight modification. Brine shrimps (Artemia salina) were hatched using brine shrimp eggs in a rectangular vessel, containing 2L of sterile artificial seawater (prepared using NaCl 38 g/L) under constant aeration for 48 h, with artificial light shone on the opposite side of the rectangular hatchery. After hatching was achieved, active nauplii were collected from the brighter side of the hatchery and used for the assay. 10 nauplii were drawn through a glass capillary and placed into vials containing 4 mL of brine solution for the control, and for the experimental groups, 10 nauplii were placed in 4 mL of brine solution. The vial for the control group contained brine solution only, whereas the vials for experimental groups contained brine solution and EEJ of various concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, and 31.25 µg/mL) with 3 duplicates per concentration. The mixtures were maintained at room temperature under constant light, and a 24 h survival larvae count was recorded. The mortality rate was determined as follows:

\[
\%\text{death} = \left(\frac{\text{Total nauplii} - \text{Alive nauplii}}{\text{Total nauplii}}\right) \times 100\%
\]

**Lethal concentration (LC50) determination**

The median LC50 in µ/mL, 95% confidence interval and slope was determined after the 24 h count using the probit statistical analysis method [34]. LC50 value of >1000 µg/mL is considered non-toxic, LC50 value 2500 µg/mL and ≤1000 µg/mL is considered weak toxic, and LC50 value <500 µg/mL is considered toxic [35].

**Effect of EEJ on castor oil-induced diarrhea in rats**

The antidiarrheal property of EEJ against castor oil-induced diarrhea was performed using a method described by Zavala-Mendoza et al. [36] with slight modifications. In brief, the rats were fasted for 18 hours before the commencement of the experiment with free access to water. A total number of 42 rats were randomly allocated into 7 groups with 6 rats per group as follows:

**Group I** was administered with normal saline (2 mL/Kg/bwt) and acted as a negative control group. **Groups II, III, and IV** were administered 100, 200, and 400 mg/kg/bwt of EEJ, respectively. **Group V** was administered loperamide, a standard antidiarrheal drug at a dose of 5 mg/kg/bwt and acted as a positive control. In a separate experiment, rats in Groups VI and VII were pre-treated with yohimbine (1 mg/kg/bwt) a selective α2 adrenergic receptor blocker and prazosin (1 mg/kg/bwt.), a selective α1 adrenergic receptor blocker respectively, 30 min before the dose of 400 mg/kg of EEJ was administered. After 1 h of various doses of EEJ and loperamide administration, 2 mL/kg/bwt of castor oil was administered orally to each rat to induce diarrhea. The animals were placed in individual cages lined with absorbent papers. The cages were then observed for 4 h for the presence of characteristic diarrheal droppings. Time for the onset of diarrhea, total number of wet droppings and semi-solid droppings was recorded for each cage during the 4 h period of observation. The time for onset was measured as the time interval between the administration of castor oil and the first appearance of wet droppings in minutes. The absence of wet droppings was considered as protection from diarrhea, and the percentage protection was calculated according to the formula described by Umukoro and Ashorobi [37] as follows:

\[
\text{NWDC-NWDT} \times 100.
\]

Where NWDC=Number of wet droppings in control group and NWDT=Number of wet droppings in treatment groups.
Effect of EEJ on gastrointestinal propulsion in rats

The effect of EEJ on gastrointestinal transit (motility) was investigated in rats using a method described by Marona and Lucchesi [38] with a slight modification. In brief, the rats were fasted for 24 h before the commencement of the experiment with free access to water. 36 rats were randomly allocated into 6 groups with 6 rats per group as follows:

Group I was given 2 ml/kg/bwt of normal saline and acted as a negative control group. Groups II, III, and IV were administered 100, 200, and 400 mg/kg/bwt of EEJ, respectively. To check whether EEJ has antimotility activity comparable to drugs with known antagonist intestinal motility, rats in Group V were given atropine sulfate (0.3 mg/kg/bwt i.p.) muscarinic receptor antagonist. In a separate experiment, rats in Group VI were pre-treated with yohimbine (1 mg/kg/bwt i.p) a selective α2 adrenergic receptor blocker, 30 min before the dose of 400 mg/kg of EEJ was administered. After 1 h of administration of the normal saline, different doses of EEJ and atropine sulfate, each animal was given 2 ml of the standard charcoal meal (10% activated charcoal suspension in 2% tween 80) and 1 ml of castor oil. After 1 hour of charcoal meal administration, each rat was sacrificed by cervical dislocation, and its abdomen was opened by midline incision. The small intestine was immediately isolated. The total length of the intestine from pylorus to caecum (total length of small intestine) and the distance traveled by the charcoal (length traveled by charcoal meal) were measured using a ruler in centimeters (cm). The peristaltic index (PI) for each rat was calculated and expressed as a percentage (%) of the distance traveled by the charcoal meal relative to the total length of the small intestine. The PI and percent inhibition relative to control were calculated according to Degu et al [39] as follows:

\[
\text{PI} = \left[ \frac{\text{LCM} - \text{LSI}}{\text{LSI}} \right] \times 100\%
\]

Where: PI=Peristaltic Index
LCM=Length traveled by charcoal meal; LSI=Total length of small intestine

\[
\% \text{ Inhibition} = \left[ \frac{(A-T)}{A} \right] \times 100
\]

Where A=Distance traveled by charcoal meal in control group and T=Distance traveled by charcoal meal in treated groups.

Effect of EEJ on castor-oil induced enteropooling

The rats were fasted for 24 h before the commencement of the experiment with free access to water. 42 rats were randomly allocated into 7 groups with 6 rats per group as follows:

- Group I was administered with 2 ml/kg/bwt of normal saline and acted as a negative control. Groups II, III, and IV were administered 100, 200, and 400 mg/kg/bwt of EEJ, respectively. Group V was administered loperamide, a standard anti-diarrheal drug at a dose of 5 mg/kg/bwt and acted as a positive control. In a separate experiment, rats in Groups VI and VII were pre-treated with yohimbine (1 mg/kg/bwt i.p) a selective α2 adrenergic receptor blocker and prazosin (1 mg/kg/bwt i.p) a selective α1adrenergic receptor blocker, respectively, 30 min before the dose of 400 mg/kg of EEJ was administered. After 1 h in each group were orally administered 2 ml/kg/bwt of castor oil. 30 min later, each rat was sacrificed by cervical dislocation, and its abdomen was opened by midline incision, and small intestine was ligated at both the pyloric and the ileocaecal junctions before it was isolated and weighed. The intestinal contents were squeezed and emptied into the graduated measuring cylinder, and the volume of contents was recorded. The intestine was reweighed without the intestinal contents, and the difference between the full and empty intestines was calculated, and percent inhibition was determined.

Statistical analysis

GraphPad Prism software was used for all analysis. The data were presented as means plus or minus standard errors of the means (SEM). One-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests was employed to compare the treatment means with control means. Student’s unpaired t-test was used to compare individual means for differences. p<0.05 was considered statistically significant.

RESULTS

Cytotoxicity of EEJ

The median LC50 after the 24 h count using the probit statistical analysis method was found to be 1225 µg/ml and was considered non-toxic since it is >1000 µg/ml.

Effect of EEJ on castor-oil induced diarrhea

Oral administration of 2 ml of castor oil produced marked copious diarrhea in all rats in the vehicle-treated control group during the 4 h period of observation. Fig. 1a and b shows that oral administration of EEJ significantly (p<0.05) and dose dependently reduced the number of wet feces induced by castor oil, with groups of 100, 200, and 400 mg/kg/bwt producing 13, 5, and 0 number of wet feces, respectively, as compared with negative control group with 21 wet feces, after a 4 h period. A dose of 400 mg/kg/bwt and loperamide (5 mg/kg/bwt) 50

Fig. 1: (a) The effect of oral administration of *Eriobotrya japonica* (EEJ) against castor oil-induced diarrhea. Values are presented as means±standard errors of the means (SEM) (n=6). *p<0.05; **p<0.01 significantly different compared with negative control group. (b) The percent protective effect of oral administration EEJ against castor oil-induced diarrhea. Values are presented as means±SEM (n=6). *p<0.01 significantly different compared with negative control group
caused complete inhibition of defecation, suggesting 100% protection, whereas EEJ at 100 mg/kg and 200 mg/kg offered 38.1% and 76.19% protection, respectively, against castor oil-induced diarrhea. Pre-treatment with yohimbine significantly reversed the protective effect of EEJ (400 mg/kg) against castor oil-induced diarrhea (52.38% vs. 100% when EEJ [400 mg/kg] was administered alone). Pre-treatment with prazosin did not significantly mitigate the protective effect of EEJ (400 mg/kg) against castor oil-induced diarrhea (97.62% vs. 100%) when EEJ (400 mg/kg) was administered alone. Fig. 1c, on the other hand, shows that EEJ at 100 mg/kg and 200 mg/kg significantly delayed the time of onset of wet feces (14±17 and 177±28 min, respectively, as compared with vehicle-treated control, 56±10 min). The presence of yohimbine caused the onset of wet feces in EEJ (400 mg/kg) treated group at the 146th min.

**Effect of EEJ on castor oil-induced gastrointestinal enteropooling**

Table 1 shows the effect of oral administration of EEJ on castor oil-induced intestinal fluid accumulation. Oral administration of EEJ significantly (p<0.05) and dosedependently (100, 200, and 400 mg/kg/bwt) reduced the volume of intestinal contents with percent inhibition of 24.37%, 45.29%, and 78.3%, respectively, compared with the negative control group. The effect of EEJ (400 mg/kg) was comparable to loperamide (75.7%). The effect of EEJ at 400 mg/kg/bwt was significantly reversed in the presence of yohimbine (45.8% vs. 79.3% when EEJ [400 mg/kg]) was administered alone. Similarly, EEJ significantly and dosedependently (100, 200, and 400 mg/kg/bwt) reduced the weight of intestinal contents with percent inhibition of 26.82%, 45.8%, and 78.41%, respectively, compared with the negative control group. The effect of EEJ (400 mg/kg) was comparable to loperamide (74.57%). The effect of EEJ at 400 mg/kg/bwt was significantly reversed in the presence of yohimbine (44.8% vs. 78.4% when EEJ [400 mg/kg]) was administered alone. The effect of EEJ at 400 mg/kg/bwt was not antagonized by prazosin.

**Effect of EEJ on castor oil-induced gastrointestinal propulsion**

Fig. 2a shows that oral administration of EEJ significantly (p<0.05) and dosedependently (100, 200, and 400 mg/kg/bwt) delayed the intestinal transit time of charcoal meal with PI of 61, 38.04, and 15.55, representing percent gastrointestinal motility reductions of 28%, 62%, and 83.92%, respectively (Fig. 2b), as compared with the negative control group with PI of 94.55. The effect of atropine at 0.3 mg/kg/bwt was more pronounced than that of EEJ at 400 mg/kg/bwt (95.92% vs. 83.92%), respectively. The propulsive inhibitory effect of EEJ at 400 mg/kg/bwt was significantly attenuated in the presence of yohimbine (64.7% vs. 83.92% when EEJ [400 mg/kg]) was administered alone.

**DISCUSSION**

Diarrhea results from an imbalance between the gastrointestinal absorptive and secretory mechanisms leading to excessive fluid and electrolytes loss in the feces. It is evident enough that diarrhea occurs as a result of increased gastrointestinal motility and secretion as well as decreased intestinal fluid absorption. Therefore, antidiarrheal drugs work by inhibiting either gastrointestinal motility or secretion or both [40]. The present study has demonstrated the potential anti-diarrheal properties of Eriobotrya japonica (EEJ) in various models of experimental diarrhea, which may be attributable to its dual antisecretory and antimotility activities.

Na+/K+/ATPase is one of the intestinal key enzymes which plays an important role in the absorption of electrolytes and water in the intestine. Any disruption or inhibition of this enzyme may cause increased intestinal fluid secretion leading to diarrhea [41,42]. Castor oil-induced diarrhea is provoked by ricinoleic acid which, in part alters the intestinal mucosa permeability to electrolytes by interfering with the normal activity of Na+/K+/ATPase resulting in abnormal intestinal absorptive and secretory mechanisms [43,44]. In the present study, EEJ significantly inhibited castor oil-induced diarrhea probably by inhibiting intestinal lipase enzymatic activity on castor oil from liberating and releasing ricinoleic acid, an active metabolite of castor oil. Therefore, lack of availability of ricinoleic acid in the intestine indirectly ensured restoration of normal absorptive capacity of the Na+/K+/ATPase with the concomitant increased intestinal absorption of Na+ along with other electrolytes and water. Studies have demonstrated the potential of various herbal extracts to directly enhance the activities of Na+/K+/ATPase followed by apparent increased intestinal absorptive and decreased secretory capacity [45]. Similar argument may explain the possible direct stimulatory effect of EEJ on the activities of Na+/K+/ATPase which might have led to the increased intestinal absorptive and decreased secretory capacity resulting in protection against castor oil-induced diarrhea. Thus, EEJ may contain phytochemicals with both direct and indirect stimulatory activity on the Na+/K+/ATPase.

**Table 1: The effect of EEJ on castor oil-induced intestinal fluid accumulation**

| Treatment                 | VIC (ml)       | % inhibition | WIC (g) | % inhibition |
|---------------------------|----------------|--------------|---------|--------------|
| Vehicle-treated control   | 5.57±0.301**   | 5.63±0.26**  | 4.12±0.287** | 26.82        |
| EEJ (100 mg/kg/bwt)       | 4.21±0.231**   | 23.37        | 3.05±0.170** | 45.8         |
| EEJ (200 mg/kg/bwt)       | 3.16±0.147**   | 43.29        | 3.12±0.162** | 78.41        |
| EEJ (400 mg/kg/bwt)       | 1.22±0.018**   | 74.18        | 1.30±0.231** | 76.86        |
| EEJ (400+yohimbine)       | 3.02±0.331**   | 45.8         | 3.10±0.314** | 44.84        |
| EEJ (400+prazosin)        | 1.26±0.032**   | 77.37        | 1.43±0.115** | 74.57        |
| Loperamide                |                |              |         |              |

Values are presented as means±SEM (n=6). *p<0.05; **p<0.01 significantly different compared with negative control group and p = 0.05; ++p<0.01 significantly different compared with EEJ (400 mg/kg/bwt). EEJ: Eriobotrya japonica; VIC: Volume of intestinal content in ml, WIC: Weight of intestinal content in g.

**Fig. 2:** (a) The effect of *Eriobotrya japonica* (EEJ) on gastrointestinal motility. Values are presented as means±standard errors of the means (SEM) (n=6). *p<0.05; **p<0.01 significantly different compared with negative control group and p<0.05 significantly different compared with EEJ (400 mg/kg/bwt). (b) The inhibitory effect of oral administration of EEJ on gastrointestinal motility. Values are presented as means±SEM (n=6)
The present study has demonstrated the potential antidiarrheal properties of ethanolic leaf extract of EEJ using various models of experimental diarrhea. The extract seems to mediate its antidiarrheal activity in part, through activation of α2-adrenoceptors. The extract may also, on the other hand, mediate its effect through modulation of the Na+/K+-ATPase and NO pathways. Therefore, extracts from EEJ, if properly and adequately standardized, may serve as a safe and cost-effective, potential therapeutic agent, with antidiarrheal properties.

The study has shed more light on the scientific basis for the use of EEJ in the treatment of diarrhea and other associated gastrointestinal disorders by some communities of the Eastern Cape Province of the Republic of South Africa.

Limitations and recommendations

The present study has established only one mechanistic pathway through which EEJ mediates its antidiarrheal effect, yet the other mentioned mechanisms are mainly based on speculations. Therefore, further investigations are warranted to establish other mechanistic pathways, through which, EEJ exerts its antidiarrheal actions.
Furthermore, the study did not carry out a phytochemical analysis of the extract as such it is imperative that future studies should focus on identification, isolation, and screening of the active compounds for their anti-diarrheal properties.

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AUTHOR’S CONTRIBUTIONS

The study was initiated and designed by Dr Kamadayaapa while all authors equally contributed to both experimental work and preparation of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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