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# Evaluation of the Analgesic Effect of Aqueous Extract of *Brugmansia suaveolens* Flower in Mice: Possible Mechanism Involved

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## Abstract

The study was conducted to test the aqueous extract of *Brugmansia suaveolens* (AE*Bs*) flowers for their antinociceptive effects in mice. In the hot plate test, a significant increase in reaction time for two doses of AE*Bs* at 60, 90, 120, and 150 min after treatment was noted. Pretreatment of animals with naloxone (5 mg/kg, intraperitoneally [IP]) left the antinociceptive effect of AE*Bs* at a dose of 100 mg/kg unaffected at 60, 90, 120, and 150 min after treatment and at a dose of 300 mg/kg at 30 min but not at 90, 120, and 150 min. In the writhing test, the AE*Bs* significantly inhibited acetic acid–induced abdominal constriction and was equally potent at both doses. Pretreatment with naloxone (5 mg/kg, IP) left the antinociceptive effect of both doses of AE*Bs* unaffected. Pretreatment with $N^G$-nitro-L-arginine methyl ester (L-NAME; 20 mg/kg, IP) caused a significant change in the number of abdominal constrictions but did not change the antinociceptive effect of AE*Bs*. Pretreatment of animals with methylene blue also did not change the effect of AE*Bs* on the number of writhing movements in mice. Flumazenil (5 mg/kg, IP) antagonized the antinociceptive effects of diazepam and also reversed the antinociceptive effect of AE*Bs*. AE*Bs* showed a depressant effect on the central nervous system, and the treatment of mice with pentobarbital combined with AE*Bs* increased the animals' sleeping time in a dose-dependent manner. These results suggest that the antinociceptive activity of AE*Bs* may be related in part to benzodiazepine receptors, although peripheral mechanisms cannot be excluded.

## Keywords

*Brugmansia suaveolens*, pain measurement, hot plate test, abdominal constriction, sleep test

According to the World Health Organization (Akerele, 1993), poverty and lack of access to modern medicines lead 65–80% of the world's population in developing countries to depend essentially on plants for primary health care. In Brazil, medicinal plants have been used extensively by people to treat bodily ailments. A case in point is *Brugmansia suaveolens* G. DON, a species from tropical South America, which is a member of the Solanaceae family. The infusion of *B. suaveolens*, popularly known as "trombeteira" or "cartucheira," has been used traditionally to induce hallucinations and to treat pain in Brazil (Oliveira, Akisue, & Akisue, 1991).

Research on the use of plants as pain relievers in traditional ethnomedicine is a productive and logical strategy in the search for new chemical substances with potential therapeutic effects (Elisabetsky, Amador, Albuquerque, Nunes, & Carvalho, 1995). Recently, we performed a study in which the antinociceptive action of an aqueous extract from the flowers of *B. suaveolens* (AE*Bs*) was assayed in different experimental models of nociception in mice (Parker et al., 2007). The purpose of this study was to investigate the involvement of the opioid system, L-arginine/nitric oxide/cyclic guanosine monophosphate (L-arginine/NO/cGMP), and the possible influence of γ-aminobutyric acid (GABA)ergic neurotransmission in the antinociceptive effect of AE*Bs* in mice.

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## Materials and Methods

### Experimental Animals

Male Swiss albino mice (25–30 g) were used in the study to minimize the effect of hormonal changes. The animals were provided by the Animal House of the Federal University of Rio Grande (FURG) and were housed in temperature-controlled rooms (20–22°C) under a 12-hr light/dark cycle (light on at 07:00). Standard rodent diet and tap water were provided ad libitum. The experimental procedures were carried out in strict compliance with the Ethics Committee rules and regulations followed by the university and in accordance with the Guide for the Care and Use of Laboratory Animals by the Brazilian School of Animal Experimentation (Colégio Brasileiro de Experimentação Animal [COBEA], 1991), Sao Paulo, Brazil. Every experiment was conducted between 09:30 and 12:30 to minimize the effects of environmental changes. The animals were placed in transparent glass observation chambers during the tests.

### Plant Material and Extraction Procedure

Fresh whole flowers of *B. suaveolens* were collected in Rio Grande, RS, and authenticated by the Department of Morphobiological Sciences (DCM, FURG). A voucher specimen was deposited at the FURG Herbarium (004025). The flowers were dried in the shade at 37°C and powdered. The powder (30 g) was extracted exhaustively in a soxhlet apparatus (Fisaton, Brazil) with distilled water (180 ml) for 6 hr. Yield of the extract was 1.2 g (w/w; 24%) of dried plant material. The aqueous extract was used for the pharmacological studies.

### Drug Preparation

Morphine (Dinomorf®), flumazenil (Flumazen®), and diazepam (Diazepam®) were obtained from the University Hospital Pharmacy at FURG. Acetic acid (0.6%) was purchased from Delaware (Porto Alegre, RS, Brazil); L-NAME, methylene blue, pentobarbital, and naloxone were purchased from Sigma (St. Louis, MO). The extract and drugs were dissolved in saline solution (0.9%) for administration.

### Evaluation of Antinociceptive Activity

*Hot plate test.* The hot plate test was used to measure response latency according to the method described previously by Eddy and Leimbach (1953), with minor modifications. In the experiments, mice were placed individually on a hot plate (Insight, Barcelona, Spain) maintained at 54 $\pm$ 1°C before receiving their pretreatments. We recorded the time that elapsed until the animal jumped or licked one of its hind paws (latency time in seconds); this time was considered the reaction time. Mice showing a pretreatment reaction time greater than 12 s were not used in the subsequent test. A cutoff time of 30 s was imposed to avoid tissue damage. Reaction time was again measured in mice 30, 60, 90, 120, and 150 min after pretreatment.

*Writhing test.* For the writhing test, mice received a 0.6% acetic acid injection (10 ml/kg; intraperitoneal [IP]) 30 min after receiving their respective treatments. The mice were observed for the number of abdominal contractions and stretches counted over a period of 25 min starting 5 min after the acetic acid injections (Koster, Anderson, & de Beer, 1959).

### Assessment of Pentobarbital-Induced Sleep Test

Thirty minutes after IP administration of AE*Bs* (100 or 300 mg/kg; 0.1 ml/10 g body weight) or saline solution (0.9 %), mice in all three groups received an IP subhypnotic dose of pentobarbital sodium (25 mg/kg). Animals were observed for loss of the righting reflex (Soulimani et al., 2001) and were considered asleep if they did not roll back when turned over. The number of sleeping animals was recorded.

### Assessment of Pharmacological Studies

*Role of opioid receptors in the antinociceptive effects of AEBs in the hot plate test.* In the hot plate test, one group, which served as the negative control group, was pretreated with saline solution (0.9%, 10 ml/kg body weight, IP). Two positive control groups were pretreated with reference drugs. One group received only morphine (10 mg/kg body weight, IP), an opioid analgesic, and the other received morphine (10 mg/kg body weight, IP) and naloxone (5 mg/kg body weight, IP), a nonselective antagonist of opioid-system receptors. Two groups were pretreated with AE*Bs*. One group received a 100 mg/kg and the other a 300 mg/kg body weight dose, IP. Two groups were pretreated with AE*Bs* in 100 and 300 mg/kg doses plus naloxone (5 mg/kg body weight, IP).

*Role of opioid receptors in the antinociceptive effects of AEBs in the writhing test.* To investigate the involvement of opioid receptors in the antinociceptive effect of AE*Bs*, mice were pretreated with saline solution (0.9%, 10 ml/kg body weight, IP) or naloxone (5 mg/kg body weight, IP) and were administered with AE*Bs* (100 and 300 mg/kg, body weight, IP), morphine (10 mg/kg body weight dose, positive control), or saline (0.9%, 10 ml/kg body weight, IP), after 30 min. The writhing test was performed 30 min later as described.

*Role of the nitric oxide-L-arginine pathway in the antinociceptive effects of AEBs in the writhing test.* To investigate the participation of the nitric oxide-L-arginine pathway in the antinociceptive effects of AE*Bs*, mice were pretreated with saline solution (0.9%, 10 ml/kg body weight, IP), L-NAME (20 mg/kg body weight, IP), an inhibitor of NO synthase, or methylene blue (20 mg/kg body weight, IP), an inhibitor of guanylate cyclase, and after 30 min, the animals were administered AE*Bs* (100 and 300 mg/kg, body weight, IP) or saline (0.9%, 10 ml/kg

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**Table 1.** Effects of an Aqueous Extract of Brugmansia suaveolens (AEBs) Flowers on Heat-Induced Pain in Mice (Hot Plate Test)

| | Latencies | | | | | |
|---|---|---|---|---|---|---|
| Treatment (mg/kg, IP) | 0 min | 30 min | 60 min | 90 min | 120 min | 150 min |
| Control | 2.31 ± 0.20 | 2.15 ± 0.18 | 4.06 ± 0.48 | 5.88 ± 0.75 | 6.42 ± 1.44 | 6.61 ± 1.44 |
| Morphine (10) | 3.1 ± 0.57 | 13.39 ± 2.79* | 19 ± 3.35* | 24.32 ± 2.94* | 17.35 ± 4.14* | 16.78 ± 3.92* |
| Morphine + naloxone (10 + 5) | 3.2 ± 0.55 | 13.47 ± 1.62* | 14.3 ± 1.92*,** | 13.02 ± 1.5*,** | 12.66 ± 2.19*,** | 11.17 ± 2.2* |
| AE*Bs* (100) | 3.73 ± 0.47 | 5.14 ± 0.64 | 13.17 ± 2.01* | 20.23 ± 3.24* | 27.03 ± 1.48* | 16.38 ± 2.2* |
| AE*Bs* + naloxone (100 + 5) | 3.18 ± 0.45 | 7.79 ± 1.03 | 14.37 ± 3.41* | 21.03 ± 2.91* | 27.17 ± 2.87* | 24.17 ± 2.73* |
| AE*Bs* (300) | 3.16 ± 0.37 | 7.73 ± 2.45 | 17.44 ±4.74* | 27.12 ± 2.88* | 20.4 ± 2.64* | 20.78 ± 2.41* |
| AE*Bs* + naloxone (300 + 5) | 3.78 ± 0.41 | 18.77 ± 2.36*,***,† | 18.2 ± 2.99* | 12.3 ± 2.09*,***,† | 7.02 ± 1.31***,† | 6.8 ± 1.24***,† |

NOTE: Mice were placed on the hot plate at 0, 30, 60, 90, 120, and 150 min after treatment. Latency values are expressed as mean ± standard error of mean (*SEM*). To the control group, only intraperitoneal (IP) saline solution (0.9%) was administered. $n = 6$ in each group. Differences between groups were analyzed by one-way analysis of variance followed by Tukey's test.
* $p < .05$ versus control.
** $p < .05$ versus morphine.
*** $p < .05$ versus AE*Bs* (100).
† $p < .05$ versus AE*Bs* (300).

body weight, IP). The writhing test was performed 30 min later as described.

*Role of benzodiazepine receptors in the antinociceptive effects of AEBs in the writhing test.* To investigate the possible influence of GABAergic neurotransmission on the antinociceptive effects of AE*Bs*, mice were pretreated with flumazenil, a benzodiazepine antagonist (5 mg/kg body weight, subcutaneously [SC]), 30 min before the administration of AE*Bs* (100 and 300 mg/kg body weight, IP). Control groups received diazepam (5 mg/kg body weight, IP) or saline solution (0.9%; 10 ml/kg body weight, IP). The writhing test was performed 30 min later, as described.

### Statistical Analysis

All results are expressed as mean ± standard error of mean (*SEM*). Statistical significance was determined using analysis of variance (ANOVA) followed by Tukey's post hoc test. Values were considered significantly different at $p < .05$.

**Table 2.** Effect of Naloxone on the Antinociceptive Effects of Morphine and an Aqueous Extract of *Brugmansia suaveolens* (AEBs) Flowers on Acetic Acid–Induced Writhing Behavior in Mice

| Treatment (mg/kg, IP) | Abdominal Constrictions (No. During 25 min) | Inhibition of Writhing (%) |
|---|---|---|
| Control | 58 ± 5.1 | – |
| Morphine (10) | 1 ± 0.52* | 98.3 |
| Naloxone (5) | 48.9 ± 9 | 15.7 |
| Morphine + naloxone (10 + 5) | 51.9 ± 3.9** | 10.5 |
| AE*Bs* (100) | 0 ± 0* | 100 |
| AE*Bs* + naloxone (100 + 5) | .1 ± 0.1* | 99.8 |
| AE*Bs* (300) | 0 ± 0* | 100 |
| AE*Bs* + naloxone (300 + 5) | 0 ± 0* | 100 |

NOTE: Values are expressed as means ± *SEM*. To the control group, only saline solution (0.9%) was administered (IP). $n = 12$ in each group. Differences between groups were statistically analyzed by a one-way analysis of variance followed by Tukey's test.
* $p < .05$ versus control.
** $p < .05$ versus morphine.

## Results

Table 1 shows the effects of the AE*Bs* (100 and 300 mg/kg body weight, IP) in the hot plate test. A significant ($p < .05$) increase in reaction time for both doses at 90, 120, and 150 min after treatment was noted. Morphine, at a dose of 10 mg/kg body weight, IP, also showed a significant increase in reaction time at 30, 60, 90, 120, and 150 min following injection. The pretreatment of animals with naloxone (5 mg/kg body weight, IP) given 30 min before injection of morphine (10 mg/kg body weight, IP) or of AE*Bs* reversed the antinociception induced by morphine at all but the 60-min time point as well as the antinociception induced by the 300 mg/kg body weight treatment with AE*Bs* at all but the 30- and 60-min time points. However, it left the antinociceptive effects of the 100 mg/kg dose of AE*Bs* unchanged at all time points after treatment. As opposed to reversing the antinociception at 30 min for the AE*Bs* (300), the naloxone actually seems to have increased it (7.73 vs. 18.77). We have no explanation for this effect.

The results presented in Table 2 show the effects of AE*Bs* (100 and 300 mg/kg body weight, IP) on the writhing response in mice in response to administration of acetic acid. AE*Bs* significantly inhibited ($p < .05$) acetic acid–induced abdominal constriction and was equally potent at both doses (100%). Morphine (10 mg/kg) significantly inhibited the writhing response. The pretreatment of animals with naloxone (5 mg/kg body

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**Table 3.** Effect of NG-Nitro-l-Arginine Methyl Ester (l-NAME) and Methylene Blue on the Antinociceptive Activity of an Aqueous Extract of *Brugmansia suaveolens* (AEBs) Flowers on Acetic Acid–Induced Writhing Behavior in Mice

| Treatment (IP; mg/kg) | Number of Constrictions (Over 25 min) | Inhibition (%) |
|---|---|---|
| Control | 58.00 ± 4.73 | – |
| L-NAME (20) | 38.25 ± 2* | 34.1 |
| AE*Bs* (100) | 0 ± 0* | 100 |
| L-NAME + AE*Bs* (20/100) | 2.75 ± 1.01* | 95.2 |
| AE*Bs* (300) | 0 ± 0* | 100 |
| L-NAME + AE*Bs* (20/300) | .25 ± 0.25* | 99.6 |
| Methylene blue (20) | 9 ± 2.90* | 80 |
| Methylene blue + AE*Bs* (20/100) | 0 ± 0* | 0 |
| Methylene blue + AE*Bs* (20/300) | .25 ± 2.00* | 99.6 |

NOTE: Values are expressed as means ± *SEM*. To the control group, only saline solution (0.9%) was administered (IP). *n* = 12 in each group. Differences between groups were statistically analyzed by a one-way analysis of variance followed by Tukey's test.
* *p* < .05 versus control.

**Table 4.** Effect of Flumazenil on the Antinociceptive Activity of Diazepam and an Aqueous Extract of Brugmansia suaveolens (AEBs) Flowers on Acetic Acid–Induced Writhing Behavior in Mice

| Treatment (mg/kg, IP) | Number of Constrictions | % Inhibition |
|---|---|---|
| Control | 58 ± 5.1 | – |
| Diazepam (20) | 8.9 ± 1.7* | 84.8 |
| Diazepam + flumazenil (20/5) | 26.5 ± 3.2** | 54.8 |
| AE*Bs* (100) | 3.1 ± 0.7* | 94.6 |
| AE*Bs* + flumazenil (100/5) | 11.9 ± 1.6**** | 79.7 |
| AE*Bs* (300) | 1.5 ± 0.6* | 97.4 |
| AE*Bs* + flumazenil (300/5) | 10.1 ± 1.7*,† | 82.8 |

NOTE: Values are expressed as mean ± *SEM*. To the control group, only saline solution (0.9%) was administered (IP). *n* = 12 in each group. Differences between groups were statistically analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's test.
* *p* < .05 versus control.
** *p* < .05 versus diazepam.
*** *p* < .05 versus AE*Bs* (100).
† *p* < .05 versus AE*Bs* (300), ANOVA and Tukey's as a post hoc test.

**Table 5.** Effect of an Aqueous Extract of Brugmansia suaveolens (AEBs) on Pentobarbital-Induced Sleep in Mice

| | Control (Saline) | AE*Bs* (100 mg/kg) | AE*Bs* (300 mg/kg) |
|---|---|---|---|
| *N* (%) of mice in which sleep was induced | 0 (0) | 3 (37.5) | 7 (87.5) |

NOTE: Mice were pretreated with saline (0.9%) or AE*Bs* (100 and 300 mg/kg) 30 min prior to treating with a subhypnotic dose of pentobarbital (25 mg/kg, IP); *n* = 8 in each group.

weight, IP) given 30 min before injection of morphine (10 mg/kg body weight, IP) reversed the antinociception induced by morphine but left the antinociceptive effect of AE*Bs* unaffected at both doses.

The pretreatment of animals with L-NAME (20 mg/kg body weight, IP), a competitive and reversible inhibitor of NO synthase, caused a significant change in the number of abdominal constrictions in mice in the acetic acid writhing test (Table 3). However, this treatment did not significantly change the antinociceptive effects of AE*Bs*. The pretreatment of animals with methylene blue, a cGMP inhibitor, significantly reduced the number of abdominal constrictions. There was no change in the effect of AE*Bs* on the number of abdominal constrictions in animals pretreated with methylene blue.

The influence of flumazenil, a benzodiazepine antagonist (5 mg/kg body weight, IP), on the antinociceptive effect of AE*Bs* and diazepam (2.5 mg/kg body weight, IP) in the writhing test is shown in Table 4. Flumazenil antagonized the antinociceptive effect of diazepam and also reversed the antinociceptive effect of AE*Bs*.

AE*Bs* showed a depressant effect on the central nervous system. The extract potentiates pentobarbital-induced sleeping. In the current study, treatment of mice with pentobarbital combined with AE*Bs* increased the number of animals sleeping in a dose-dependent manner, indicating pharmacological effect (Table 5).

## Discussion

In our tests, AE*Bs* flowers induced nociception in mice. This study used chemical- and thermal-induced nociception in mice, namely the hot plate test and the acetic acid–induced writhing responses. Our previous data suggest that the antinociceptive effect of AE*Bs* may involve supraspinal and spinal components, as demonstrated by the results of the hot plate test, as well as local peritoneal receptors, as demonstrated by the results of the writhing test (Parker et al., 2007).

In the current study, the antinociception elicited by AE*Bs* seems to be independent of the activation of an important endogenous analgesia system, namely the opioidergic system. In the hot plate test, it is clear that the extract caused a considerable prolongation of latency times, indicative of centrally mediated activity. However, treatment of mice with naloxone, a nonselective opioid antagonist, failed to interfere with AE*Bs*-induced antinociception. In contrast, the antinociceptive action of morphine was reversed by naloxone. In the writhing test, the antinociception elicited by AE*Bs* seems to be also independent of the activation of the opioidergic system. The treatment of animals with naloxone also failed to interfere with AE*Bs*-induced antinociception when assessed in this model. However, the antinociceptive action of morphine was, again, reversed by naloxone.

The involvement of NO in modulating pain mechanisms has been well documented (Duarte, Lorenzetti, & Ferreira, 1990; Tseng, Yu, & Pieper, 1992), and a great number of reports have attributed NO's influence on pain perception to its ability to influence nociceptive processing in both the peripheral and the central nervous systems (Duarte et al., 1990; Fidecka &

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Lalewicz, 1997). Using L-arginine/NO/cGMP cascade activators and/or inhibitors as pharmacological tools, researchers have reported that NO plays both nociceptive and antinociceptive roles in the L-arginine/NO/cGMP pathway in peripheral tissues (Kawabata, Umeda, & Takagi, 1993).

In this study, L-NAME, a competitive and reversible NO synthase inhibitor, failed to affect the antinociceptive effect of AE*Bs*. The decrease in endogenous NO within the peripheral tissues caused by L-NAME was not sufficient to influence the antinociception induced by AE*Bs*. In fact, the L-NAME treatment itself, similar to a study reported by Abaçioglu, Tunçtan, Akbulut and Cakici, (2000), induced antinociception in acetic acid–induced nociception, significantly decreasing writhing episodes induced by acetic acid. This finding was in contrast with the observation made by Zakaria, Sulaiman, Somchit, Jais, and Ali (2005) on the ability of L-NAME to affect the nociceptive threshold induced by acetic acid.

This study also revealed the ability of methylene blue, an inhibitor of the cGMP pathway, to act as an antinociceptive agent on its own, which is concomitant with the reports published by Abaçioglu et al. (2000) and Zakaria et al. (2005). Moreover, pretreatment with methylene blue did not reverse the antinociceptive effect of AE*Bs*.

It has been reported that benzodiazepine has intrinsic analgesic activity (Morgan, Depaulis, & Liebskind, 1987; Oliveira & Prado, 1994; Rodgers & Shepherd, 1989; Shah & Treit, 2004). Additionally, diazepam may reduce the volume of acute inflammatory paw edema in rats (Lazzarini, Malucelli, Muscara, de Nucci, & Palermo-Neto, 2003; Lazzarini, Malucelli, & Palermo-Neto, 2001). A direct antinociceptive effect of benzodiazepines has been demonstrated using the writhing test (Sierralta & Miranda, 1992). Using a model of arthritic pain, Fernández-Guasti, Reyes, Martinez-Mota, and López-Munoz (2005) analyzed the influence of nociception on basal levels of anxiety-like behavior. They showed that diazepam is able to induce antinociceptive action in mice in the writhing test. This effect is related to benzodiazepine receptors because the antinociceptive action of diazepam was antagonized by flumazenil. The results of the current study suggest that the antinociceptive effect of AE*Bs* was due to a GABAergic system mechanism.

A variety of chemical structures have been isolated from the leaves and seeds of *B. suaveolens* G. DON such as tropanic alkaloids. Scopolamine is the major alkaloid present in the species, but hyoscyamine and atropine are also present (Schenkel, Zaninnin, Mentz, Bordignon, & Irgang, 2004). *R*-(+)-hyoscyamine has been found to exert antinociceptive and nootropic action in rodents (Ghelardini, Galeotti, Romanelli, Gualtieri, & Bartolini, 2000). The pharmacological effects of scopolamine are even more diverse and so are the indications for its use. Additionally, its action on the central nervous system is much more pronounced, with sedation at low doses and the possibility of disorientation and hallucinations with higher doses (Grynkiewicz & Gadzikowska, 2008). Its clinical applications include obstetrical analgesia (Christen, 2000; Renner, Oertel, & Kirch, 2005).

Preliminary pharmacological screening involving the administration of AE*Bs* to rodents revealed a low toxicity for this plant (Sinnott Silva, Abreu, Argoud, Silva, & Almeida, 1995). In the current study, doses of *B. suaveolens* crude aqueous extract (100 and 300 mg/kg body weight, IP) produced a significant increase in the hypnotic effect induced by pentobarbital in a dose-dependent manner, suggesting a probable sedative activity.

As a whole, the results herein presented suggest that AE*Bs* displays considerable antinociceptive potency. The evaluation of affinity to the different central receptors using the antagonists of these receptors showed that this antinociceptive activity may be related in part to benzodiazepine receptors, although peripheral mechanisms cannot be ruled out. Therefore, *B. suaveolens* could be beneficial in the control of pain. The current study supports the folk medicinal use of this plant.

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## Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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