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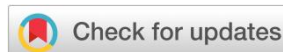
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Research Article

Evaluation of Analgesic Activity of *Cestrum nocturnum* Leaves Extract on Wistar Albino Rats

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Abstract

Cestrum nocturnum, belonging to the Solanaceae family, has traditionally been used for the management of many medical disorders. The purpose of this study was to determine the analgesic effects of the ethanolic extract obtained from the leaves of *Cestrum nocturnum* on Wistar albino rats. The acetic acid-induced writhing assay and the tail flicking experiment were two methods for standardizing the extract's analgesic properties. The findings demonstrated that the leaf extract of *Cestrum nocturnum* effectively alleviated pain in the stated tests. This indicates that it has pain-relieving benefits. Furthermore, the extract was subjected to phytochemical screening, which detected the existence of many bioactive constituents, including flavonoids, terpenes, and alkaloids. These substances may clarify the pain-relieving and anti-inflammatory effects that were reported. The results show that the ethanolic extract from *Cestrum nocturnum* leaves can relieve pain and reduce inflammation, which backs up its traditional use for these purposes in folk medicine. Further research is needed to determine the specific mechanisms by which these bioactive compounds exert their effects on pain and inflammation.

KEYWORDS: *Cestrum nocturnum*, antioxidant, flavonoids, analgesic activity, ethanopharmacology, pain.

INTRODUCTION

Pain is a warning signal that functions as a fundamental protective mechanism. Although it can cause discomfort and anxiety, in certain instances it can become unbearable and incapacitating ¹. Pain is commonly characterized as an unpleasant combination of sensory and psychological sensations that is linked to real or potential harm to tissues or discussed in connection with such harm ². Usually, it is an immediate reaction to a harmful occurrence that impacts the tissues of the body, such as damage, inflammation, or cancer. Two primary categories of pain that are widely recognized as legitimate are nociceptive sensations and neuropathic pain. Nociceptive pain, also referred to as responsive pain, is the sensation of pain resulting from harmful stimuli, such as extreme temperatures, physical trauma, or chemical irritation ³. On the other hand, neuropathic pain can occur due to damage to neuronal structures in the peripheral and central nervous systems.

Pyrexia is a term that originates from the Greek word "pyros," which translates to "fire" or "catching heat." It is the accurate medical term used to indicate an increased body temperature ⁴. Consequently, both of these names can be utilized efficiently. Fever is defined as a controlled elevation in internal temperature that surpasses the normal range. The standard limit for healthy body temperature when tested directly is 36.7°C to 37°C. Rectal temperatures often exhibit a 0.6°C increase compared to oral temperatures, while axillary temperatures generally show a 0.6°C decrease ⁵. Skin temperatures, on the other hand, are normally lower than oral temperatures but might vary based on the specific measuring technique used. Fever is a sophisticated and adaptable reaction of the body to different immunological challenges, whether they are caused by infections or non-infectious factors ⁶.

Analgesics are medicines used to relieve pain, while antipyretics are substances used to lower fever. Pain and fever usually occur due to a secondary pathological

condition and are followed by the release of inflammatory substances such as prostaglandins, cytokines, and interleukins. Thus, the treatment of these illnesses could be characterized as 'symptomatic'. After effectively managing the underlying disease, symptoms of fever and discomfort frequently subside ⁷.

Medicinal herbs have been widely used for pain relief and fever treatment, gaining popularity throughout the years. Non-steroidal anti-inflammatory medicines (NSAIDs) and corticosteroids, along with other medications, have shown varying degrees of effectiveness in managing and treating the condition. However, these medications frequently lead to unfavorable side effects and possess a restricted therapeutic scope. Hence, it is imperative to explore novel analgesic and analgesic-blocking drugs derived from conventional medicines ⁸.

Cestrum nocturnum, often known as a night-blooming jasmine, which is a plant from the Solanaceae family commonly seen in gardens (Figure 1). It has a historical record of being used in traditional medicine to address many health conditions. Previous studies have shown that the therapeutic leaves of *Cestrum* species possess noteworthy bioactive properties, including toxicity and antiepileptic effects. However, the analgesic qualities of *Cestrum nocturnum* leaf have not been thoroughly researched ⁹. The aim of this study is to evaluate the analgesic properties of an extract of leaves obtained through *Cestrum nocturnum* in albino Wistar rats.



Figure 1. *Cestrum nocturnum* leaves

MATERIAL AND METHODS

Materials

The chemicals used in this experiment include ethanol, methanol, 2,2-diphenyl-1-picrylhydrazyl, ascorbic acid, diluted hydrochloric acid, ethyl acetate, picric acid, and petroleum ether. The solvents used in the analytical procedures were obtained from various suppliers, including Chhattisgarh Distilleries Ltd, Merck Life

Sciences, Himalayan Laboratory Ltd, and Loba Chime Pvt. Ltd. A control was administered using diclofenac sodium 500 mg. The experiment utilized a variety of chemicals and tools, including distilled water, methanol, ferric chloride solution, Millions reagent, α -naphthol solution, sulfuric acid, Benedict reagent, sodium hydroxide, ethyl acetate, and ammonia solution.

Collection and authentication of plant materials

The leaves of the plant *Cestrum nocturnum* L. (family Solanaceae) were obtained from the herbal garden of Govt. Ayurveda College, Raipur, Chhattisgarh. The experts at the Dravya Guna Ayurvedic College department in Raipur, Chhattisgarh, successfully identified the plant specimen. The voucher specimen number **126578** was assigned to it.

Pre-processing of plant material

The leaves were afterwards exposed to air drying in a shaded environment at an ambient temperature of 30 °C over a period of 14 days to avoid the deterioration of important chemical components. After this duration, the dehydrated leaves were crushed into a coarse powder using a pestle and mortar to make the procedure more convenient.

Experimental animals

This study utilized adult Wistar rats weighing between 200 and 250 grams. The animals were acquired from the animal facility located at the Columbia Institute of Pharmacy in Takari, Raipur, Chhattisgarh, India. The mice were housed and cared for in regulated laboratory circumstances. The requirements included maintaining a precise temperature of 21.5 °C, a humidity level of 60 °C, and a light/dark cycle of 12 hours. They were given unrestricted access to food (in the manner of regular pellets) and water at all times. The Institutional Animal Ethics Committee (688.02/C-CPCSEA) at the Columbia Institute of Pharmacy undertook a comprehensive assessment of the experimental techniques and protocols used in this study. Their strict compliance with the principles set by the Institutional Animal Ethics Committee (IAEC), as stated in proposal number NCP/IAEC/NO: 2023-24, was praiseworthy.

Extraction

The powdered dried leaves of *Cestrum nocturnum* (Figure 2) was meticulously sifted using a number 20 sieve to achieve a uniform texture. The leaf powder was subjected to a defatting procedure using petroleum ether as the solvent (60–80 °C) for a period of 72 hours to eliminate any fatty compounds that were present. Subsequently, the powder was extracted with ethanol as a solvent (60–70 °C) in a Soxhlet extractor for duration of 36 hours. The ethanolic extract was made, filtered using Whatman filter paper, and then dried using a rota vapor. The sample was meticulously placed in an air-tight container to be kept in a freezer at a temperature range of 2°C–8°C until further use in the experiment.

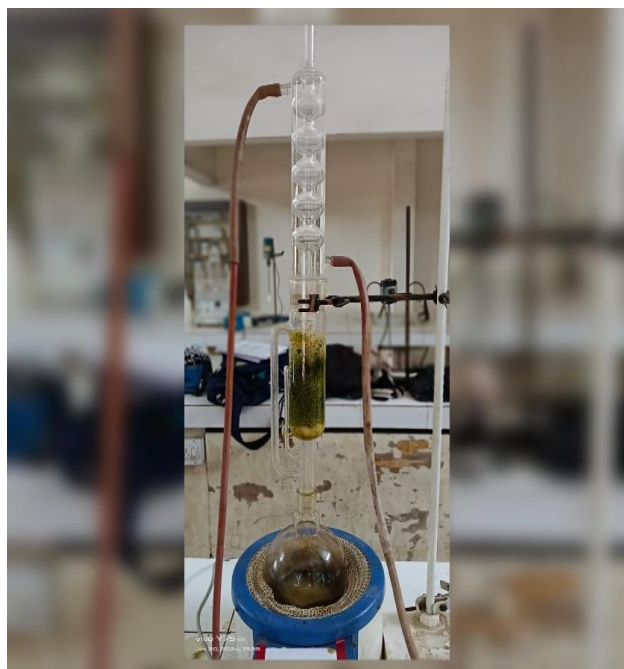


Figure 2. Extraction of *Cestrum nocturnum* leaves using Soxhlet apparatus

Preliminary qualitative phytochemical analysis

A preliminary investigation was performed to identify the existence of various secondary compounds in the ethanol extracted form from *Cestrum nocturnum* leaves. The screening was performed using standard laboratory procedures¹⁰.

Total Phenolic Content (TPC)

The Folin-Ciocalteu method, as outlined by Chouhan et al., was employed to measure the overall phenolic content. The UV-spectrophotometer was employed to measure the absorption of both the standard gallic acid and each sample at a fixed wavelength of 765 nm. The samples were analyzed to determine their total phenolic content (TPC), which was expressed as milligrams of the equivalent of gallic acid (GAE) per gram of dried extract (11).

Total Flavonoid Content (TFC)

The extract was examined to determine the total flavonoid concentration through the aluminum chloride method, as outlined by Chouhan et al. The UV spectrophotometer was used to measure the absorbance at a wavelength of 510 nm, and the results were expressed in milligram per grams of dried extract. Quercetin equivalents (QUE)¹¹.

Antioxidant activity

The DPPH test was utilized to evaluate the antioxidant capacity of various extracts by measuring their ability to

scavenge free radicals. The reduction in absorbance from the solution of DPPH at 517 nm following the introduction of the antioxidant was quantified. A solution was made by dissolving ascorbic acid in DMSO to obtain a concentration of 10 mg/mL¹¹.

$$\% \text{ Scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control refers to the absorbance measurement of the control sample.

Abs sample represents the absorbance of the experimental sample.

Acute toxicity test to determine a specific dosage

Acute oral toxicity tests were conducted using Wistar rats, following the guidelines outlined in the OECD 423 Regulations. Rats were administered a single dose of 2000 mg/kg, and their reaction was observed for a duration of 14 days. The ethanolic solution of *Cestrum nocturnum* leaves demonstrated no signs of toxicity, changes in behaviour, or death in rats, even when administered at a dose of 2000 mg/kg body weight, thus showing its safety. Animals that received an ethanol extract of *Cestrum nocturnum* leaves experienced consistent gains in body weight and maintained normal food intake throughout the whole trial period. The LD50 of the extract, as evaluated in acute toxicity experiments on rats, was found to be greater than 2000 mg/kg. According to the recommendations of the OECD 423, this categorizes it as the 5th category in the GSH categorization. For all following studies, two doses of extracts (200 and 400 mg/kg per day) was given.

Acetic Acid-Induced Mouse Writhing Test

The samples were evaluated for their pain-relieving effectiveness utilizing an acetic acid triggered writhing technique in rats. This method entails the introduction of acetic acid into the peritoneal cavity to elicit pain sensations in the subjects of the experiment (Figure 3a and 3b). Any widely-used NSAID medication can be employed as a form of positive control. The current experiment employed diclofenac sodium. The Wistar rats were divided into four groups, each containing six individuals. Group I was designated as the untreated group and was administered acetic acid intraperitoneally. The presence of writhing was seen and documented. Groups II, III, and IV received diclofenac sodium to the rats at a dose of 10 mg/kg, along with the extract at doses of 100 and 200 mg/kg. Following a time period of fifteen minutes, the acetic acid was administered, and the onset and magnitude of the writhing response were monitored⁶.

$$\text{Inhibition \%} = \frac{\text{Number of writhes (Control)} - \text{Number of writhes (Treatment)}}{\text{Number of writhes (Control)}} \times 100$$



Figure 3(a) and 3 (b). Depicts the administration of drugs (extracts) during experimental study

Tail immersion method

The analgesic effect was evaluated in albino Wistar rats utilizing the proximal immersion method. The animals were placed in individual restraining enclosures, allowing their tails to remain unrestricted. The final 5 cm section of the tail was marked and placed in a water bath with a temperature of 55 ± 0.5 °C that was carefully regulated. Detaching the appendage from the heated liquid showcased the pain-relieving effect. The response time was measured using a chronometer. Each animal served as a control. The average of the two figures indicated the initial reaction time. The reaction times of the groups were recorded at 0, 30, 60, 90, and 120 minutes. The immersion process had a time limit of 15 seconds. The assessment of reaction time was carried out ¹².

Statistical analysis

The data was presented as the average $\pm$ the standard deviation (SD) of triplicate the samples, with $n = 3$. The

study employed a one-way analysis of variance (ANOVA) utilizing the statistical software SPSS version 20. Statistical significance was established when the p-value was less than 0.05. The graphical depiction was generated utilizing Microsoft Excel 2007.

RESULTS

Extract's percentage yield

The ethanolic extract obtained from 100 g of crude *Cestrum nocturnum* leaves has a percentage yield of 10.25% w/w.

Preliminary phytochemical screening results

Table 1 presents the findings of a qualitative phytochemical investigation carried out on the ethanolic solution of *Cestrum nocturnum* leaves. The investigation effectively detected an existence of saponins, tannins, proteins, carbohydrates, reducing sugars, glycosides, flavonoids, and alkaloids ¹³.

Table 1 displays the findings of the analysis of plant compounds in the ethanolic solution of *Cestrum nocturnum* leaves.

Analysis of the phytochemicals in the extract of <i>Cestrum nocturnum</i> leaves		
Alkaloids	Dragendorff's Test	+++
	Wagner's Test	+++
	Mayer's Test	+++
	Hager's Test	+++
Carbohydrates	Fehling's Test	+++
	Molisch Test	+++
	Benedict's Test	+++
Proteins	Biuret Test	+++
	Millon's Test	+++

Tannin	FeCl ₃ Test	+++
Flavonoids	Shinoda Test	+++
Glycosides	Legal's Test	+++
	Borntrager's Test	+++
Steroids	Liebermann Burchard Test	+++
	Salkowski Test	+++

Key: +++ = detected

A quantitative and qualitative phytochemical screening was conducted on the ethanolic solution of *Cestrum nocturnum* leaves. The investigation identified various bioactive constituents, such as flavonoids and phenolics, that possess the capacity to neutralize free radicals. Reductones act as antioxidants by transferring hydrogen atoms to a free radical. This phenomenon is commonly

linked to the declining capacity of chemicals, which can be used as a dependable indicator of their efficacy as antioxidants. The presence of a significant quantity of reductone in the ethanolic solution of *Cestrum nocturnum* leaves indicates its potential as a highly effective antioxidant ¹⁴.

Table 2 presents the quantitative measurements of the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH assay) of the ethanolic extract derived from *Cestrum nocturnum* leaves.

Quantitative analysis of ethanolic extract of <i>Cestrum nocturnum</i> leaves		
Total phenolic content (mg/gm GAE)	3.12 ± 0.08	
Total flavonoid content (mg/gm QE)	2.25 ± 0.15	
Antioxidant activity (DPPH assay) (IC ₅₀) ^a (µg/ml)	75.22 ± 0.28	Ascorbic acid (Control) 82.09 ± 0.85

Results are expressed as mean values ± SD (n=3).^aIC₅₀ Concentration of sample required to scavenge 50% of free radicals. IC₅₀ values are expressed as µg dry extract/ml (µg/ml). SD: Standard deviation; DPPH: 2,2'-diphenyl-1-picrylhydrazyl; IC₅₀: Half maximal inhibitory concentration; *Cestrum nocturnum*

Analgesic Activity

Acetic Acid-Induced Writhing Test

Injecting acetic acid into the peritoneal region of mice, without any experimental treatment, also induced the writhing reflex. The given extract, at a dosing range of 100-200 mg/kg, demonstrated a significant and dose-

dependent reduction in the total quantity of writhes recorded in the mice. The highest impact was noted at a dose of 300 mg/kg, leading to a decrease of 91.6%. The administration of diclofenac sodium at a dosage of 10 mg/kg resulted in a significant reduction of 5.9% and 14.0% in the therapeutic effects of the ethanolic solution of *Cestrum nocturnum* leaves (Table 3).

Table 3 presents the impact of the ethanolic extract of *Cestrum nocturnum* leaves on the Acetic Acid-Induced Writhing Test in rats.

Treatment	Dose (mg/kg)	No of writhes	% inhibition
Distilled water (Group - I) (ml/kg)	10	22.8 ± 1.6	--
Diclofenac sodium (Control)	10	10 ± 0.2	90 %
Group - II	200	19.8 ± 0.4	65.2%
Group - III	300	12 ± 0.8	91.6%
Group - IV	400	16 ± 0.7	70.5%

Values are mean ± SEM. n=5, p < 0.01, p < 0.001 vs. control; p < 0.001 vs *Cestrum nocturnum* leaves (100 mg/kg, 200 mg/kg and 400 mg/kg) (one way ANOVA followed by student t test test).

Tail flick analgesic test results

Table 4 presents the latency of mice's tail movement in response to submerging their rear ends in hot water maintained at a uniform temperature of 50 °C. The mice exhibited a statistically significant increase in the duration of tail flicking when administered Diclofenac sodium at a dosage of 10 mg/kg and *Cestrum nocturnum* at dosages of 100, 200, and 400 mg/kg, as compared with the negative control group that received distilled water. The mean response time exhibited variation across multiple time periods (0, 30, 60, 90, and 120 minutes afterward post-treatment). The rats that received diclofenac sodium at a dose of 10 mg/kg was the longest response time compared to all other time periods. The peak reaction time occurred in the 90th

minute of the session, with a mean time of 35.6 seconds. Administering *Cestrum nocturnum* at a dosage of 200 mg/kg caused a significant change in reaction time, beginning 60 minutes after treatment and lasting till 90- and 120-minutes post-treatment. The mean response time at 60, 90, and 120 minutes in length was 15.7, 28.33, and 16.1 seconds, accordingly Administration of 300 mg/kg of extract caused noticeable responses at 30, 60, and 90 minutes following treatment. The average duration of the responses was 10.28, 18.75, and 19.46 seconds after 60, 90, and 120 minutes, respectively. Administering a dose of 400 mg/kg of the extract resulted in a significant increase in reaction time only in the 90th minute, with a mean response time of 14.53 seconds.

Table 4 presents the findings from a study of tail flick analgesic assay effects of ethanolic extract obtained from the leaves of *Cestrum nocturnum*.

Group	Dose (mg/ kg)	Reaction time (secs)				
		0 mins	30 mins	60 mins	90 mins	120 mins
Distilled water	10	4.2 ± 0.12	4.26 ± 0.18	4.14 ± 0.79	4.25 ± 0.83	4.31 ± 0.13
Diclofenac sodium	10	5 ± 0.58	15 ± 0.5	22.4 ± 0.07	22.12 ± 0.2	23.1± 0.14
Group -I	200	6.7 ± 0.82	10.28 ± 0.67	18.75 ± 0.54	19.46 ± 0.61	17.13 ± 0. 66
Group -II	300	6.45 ± 0.06	13.11 ± 0.3	15.28 ± 0.68	17.75 ± 0.16	16.32 ± 0.15
Group -III	400	6.42 ± 0.09	10.55± 0.78	12.59 ± 0.08	14.53 ± 0.49	15.42 ± 0.67

The values are represented as the average ± standard deviation (N = 5). The p-value was found to be less than 0.05 when comparing to distilled water using a one-way ANOVA followed by Tukey's multiple comparison test.

DISCUSSION

Table 1 and Table 2 display the findings from the analysis of the chemical components in the ethanolic solution of *Cestrum nocturnum*, using both qualitative and quantitative methods. The initial phytochemical investigation and quantitative analysis of antioxidants unveiled the presence of saponins, tannins, proteins, carbohydrates, reducing sugars, glycosides, flavonoids, phenolics, and alkaloids ¹⁵. The leaf extract also has significant antioxidant properties, consistent with the results obtained for phenolics and flavonoids ¹⁶. The finding is similar to the secondary compounds that were detected by researchers in other *cestrum* species, specifically *Cestrum dirinium* ¹⁷. The properties of a plant's secondary metabolites significantly influence the particular metabolic processes in which it engages. A wide range of phytochemicals, such as triterpenes, flavonoids, alkaloids, steroids, tannins, and glycosides, have been identified as the causative agents for the different biological effects observed in plant extracts. Previous research has shown that plant compounds, such as tannins, alkaloids, flavonoids, and phenolic acids, possess analgesic, anti-inflammatory, and antipyretic activities when tested on animals in experiments ⁹. These substances are accountable for these pharmacological effects. Flavonoids are suggested

to possess analgesic properties by influencing opioid pathways ¹⁸. Flavonoids work by inhibiting the oxidation process of arachidonic acid. This decreases the levels of prostaglandins, hence relieving fever and discomfort ⁴. Flavonoids hinder the production for a substance called tumor necrosis factor, a type of cytokine that stimulates the production of PGE2, an essential mediator of pain and fever ³. Chemical compounds like alkaloids, terpenoids, and steroid hinder the synthesis of prostaglandin synthase. This leads to an augmented synthesis of PGE2. The analgesic effects of the *Cestrum nocturnum* extract were ascribed by the abundance of flavonoid and alkaloids in the extracts ¹². They accomplish this by interacting directly or indirectly through receptors in the body inside of cells, intracellular facilitators, and nucleic acids that are involved in these processes.

The ethanol-based extract of *Cestrum nocturnum* exhibited significant analgesic effects in both the acetic acid and tail flick models.

The test specimens were evaluated for their analgesic efficacy utilizing an acetic acid triggered writhing method, a widely employed methodology for detecting peripheral effects. The findings of our investigation unequivocally demonstrated that the extracts derived from *Cestrum nocturnum* had analgesic properties that

specifically targeted the nerves in the peripheral area, hence alleviating writhing. These observations suggest that *Cestrum nocturnum* holds the potential to serve as an alternative source for currently available analgesic medicines. Pain is caused by the onset of localized inflammatory sensations, as explained by the peripheral nociception hypothesis. Following inflammation, prostaglandins are synthesized via the cyclooxygenase pathway, whereas leukotrienes are synthesized via the lipoxygenase system¹⁹. Prostaglandins, particularly prostacyclin (PGI₂) and to a lesser extent prostaglandin E, are thought to induce discomfort and abnormal contractions in mice, resulting in a behaviour referred to as 'writhing'. The phytoconstituents present in *Cestrum nocturnum* extracts can inhibit the synthesis of lipoxygenase and/or cyclooxygenase enzymes, hence impacting the formation of PGE₂²⁰. As a result, the remodelling process of the nociceptor is hindered, causing a decrease or complete absence of pain sensation.

Pain perception is governed by a complex interplay of several processes, such as neuro opiates, dopamine-producing, downward noradrenergic, and serotonergic pathways. The tail flick simulation is commonly acknowledged as an extremely painful model¹. The tail withdrawal reaction in mice predominantly signifies any signs of some sort of central acting analgesic mechanism, while medications that operate peripherally are observed to have little impact upon the heat-induced pain response. The pain threshold in this experiment was shown to increase when *Cestrum nocturnum* extract was given at doses of 200-400 mg/kg. This suggests that the extract of the leaves may have the ability to reduce pain that is controlled by the central nervous system. This could be attributed to its influence on any of the systems involved in this specific type or ache, including the opioid system or endocannabinoid systems. Additional investigation is required to comprehensively comprehend the processes through which the extract of *Cestrum nocturnum* produces its pain-relieving benefits in this particular model.

CONCLUSION

The present study has confirmed the traditional uses of *Cestrum nocturnum* and establishing how leaf extract of the plant possesses analgesic properties. Considering its analgesic, anti-inflammatory, and antipyretic properties, it has the potential to be more advantageous in the treatment of inflammation, pain, and fever, including dengue fever. Medications can alleviate inflammation through several routes. These effects include actions similar to corticosteroids, the release of naturally produced glucocorticoids, participation in the creation of the cytokines or other compounds related to inflammation. Further investigation is necessary to understand the mechanisms that help *Cestrum nocturnum* leaves demonstrate their analgesic properties. This will enable the advancement of more effective and targeted treatments for a variety of diseases. Moreover, it is crucial to conduct clinical trials to evaluate the safety and efficacy of using this specific

plant species is a feasible alternative for pain management in the field of medicine.

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Conflict of Interest: The authors declare that they no conflict of interest.

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