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Research Article

Enhancing Intranasal Delivery and Bioavailability of Dihydroergotamine Utilizing Chitosan Nanoparticles

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Objective. Dihydroergotamine (DHE) is used for acute migraine treatment. Oral DHE is extensively metabolized; therefore, it must be given by a nonoral route. The aim of this study was to investigate the potential use of chitosan nanoparticles as a system for improving the systemic absorption of dihydroergotamine (DHE) following nasal administration. *Methods*. DHE-loaded chitosan nanoparticles (CS-NPs) were prepared by a modified ionotropic gelation method with sodium tripolyphosphate. The resulting nanoparticles were evaluated for size, drug loading, and *in vitro* release. DHE was administered at a dose of 0.5 mg/kg to male Sprague–Dawley rats intravenously, as an intranasal solution, or intranasal nanoparticles ($n = 3$ in each group). A special surgical procedure was performed to ensure that the drug solution was held in the nasal cavity. Blood samples were collected at appropriate times for 90 min. An HPLC-fluorescence detection method was employed to determine DHE in the plasma. *Results*. DHE chitosan nanoparticles with 20% loading had 95 ± 13% encapsulation efficiency and a particle size of 395 ± 59 nm. *In vitro* DHE release studies showed an initial burst followed by a slow release of DHE. DHE intranasal nanoparticles demonstrated significantly increased absolute bioavailability (82.5 ± 12.3%) over intranasal DHE solution administration (53.2 ± 7.7%). *Conclusion*. Taking in consideration the limitations of delivering DHE, the results of the present study demonstrate that DHE CS-NPs have a great potential for nasal DHE administration (55% increase in bioavailability) compared to intranasal solution with effective systemic absorption.

1. Introduction

Migraine is a chronic headache disorder with a major impact on the lifestyle of patients. It has been found to be the second leading cause of disability in the United States regarding "impaired quality of life, substantial lost productivity, and high-economic costs" [1]. Approximately 17% of women and 6% of men in the United States were reported to suffer from migraines [2]. One study in Jordan found that around 7.7% of adults experience migraines [3]. Migraine patients usually prefer medications with rapid onset and fast pain relief [4–6]. Furthermore, many migraines present with nausea, which makes swallowing oral medications difficult, requiring alternative dosage forms like intranasal sprays [7].

Dihydroergotamine (DHE) ((5′α)-9,10-dihydro-12′-hydroxy-2′-methyl-5′-(phenylmethyl)-ergotaman-3′,6′,18-trione) (Figure 1) is a chemical derivative of an ergopeptine core alkaloid. It binds to serotonin 5-hydroxytryptamine 1 ($5HT_1$) receptors, making it a highly effective medication for the treatment of migraine [8]. DHE has been found to be helpful with rebound headaches, chronic daily headaches, menstrual migraines, and in patients not responding to triptan treatment [9, 10].

The drug has low oral bioavailability. Oral DHE is extensively metabolized by the first-pass effect; therefore, it must be given by a nonoral route. Furthermore, it has incomplete and inconsistent drug passage across the gastrointestinal mucosa, making it suboptimal for clinical use [11].

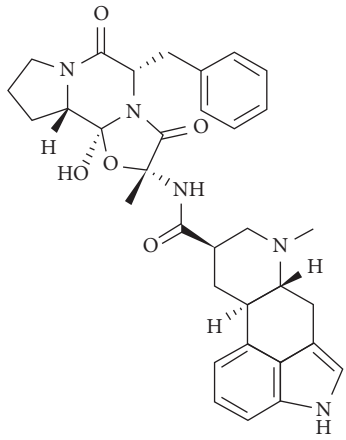

Figure 1: Structure of dihydroergotamine (DHE).

Parenteral DHE requires healthcare personnel to administer, and it is challenging to self-administer. This demands the development of innovative strategies to overcome these difficulties. The nasal route is an attractive modality to enhance therapeutic efficacy and to reduce the extent of the first-pass effect. Furthermore, alternative drug delivery systems have been proposed such as inhalations, microneedles, and injections, which bypass the liver and are more rapidly absorbed [10, 12–15].

The use of intranasal dosage forms to treat migraines was investigated with DHE [16–20], as well as sumatriptan [21–23], lidocaine [24–27], and civamide [28]. Intranasal drug delivery shows promise for simple and noninvasive medication administration while avoiding hepatic first-pass metabolism, thus improving bioavailability [29–31]. More bioavailable drugs result in decreased dose-related side effects and an improved safety profile. The rapid onset of action with intranasal administration also offers benefits for quick pain relief and missed doses. Intranasal delivery of DHE solution increases bioavailability over oral dosage forms [32], but in order to further improve drug delivery and bioavailability, intranasal migraine medications have been formulated using mucoadhesive materials with promising results [33–35].

Chitosan is a mucoadhesive polysaccharide derived from partially acetylated chitin [36] that has been often prepared as nanoparticles to improve intranasal delivery of drugs [37–39]. It is mucoadhesive and has been shown to enhance drug permeability across membranes [40], thus making it a promising candidate for intranasal drug delivery.

The marketed nasal DHE spray has low systemic bioavailability (32%) [41] and intersubject differences in self-administration, along with reported spillage [42] which can lead to drug loss, disturbed taste, and abdominal pain as it runs out of the upper lip or down the back of the nasopharynx, leading to suboptimal therapeutic effects [42–44], and rhinitis, which is a common adverse event [41].

The aim of our work includes the formulation of DHE in chitosan nanoparticles for intranasal application to enhance systemic absorption. To our knowledge, this is the first study to investigate DHE intranasal delivery with nanotechnology in an anesthetized rat model.

2. Materials and Methods

2.1. Materials. Dihydroergotamine was purchased from Medisca (Plattsburgh, New York, USA). Low molecular weight chitosan (40,000 Da), trifluoracetic acid, triethylamine, acetonitrile, and tertiary butyl L methyl ether solutions were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Sodium tripolyphosphate, potassium diphosphate, and glacial acetic acid were purchased from BBC Chemicals (Torre Boldone BG, Italy). Heparin sodium was purchased from B. Braun (Bethlehem, Pennsylvania, USA). Diethyl ether was from Alpha Chemika (Mumbai, India). Xylazine, ketamine, and phosphoric acid were from MERCK (Rahway, New Jersey, USA).

2.2. Preparation of Chitosan Nanoparticles. Chitosan nanoparticles (CS-NPs) were prepared based on a modified ionotropic gelation method of chitosan with tripolyphosphate (TPP) polyanions [45]. Chitosan (200 mg, 0.20% w/v) was dissolved in 100 mL of 3% (v/v) acetic acid with the pH adjusted to 4.7 using 10 N NaOH. Then, TPP aqueous solution (0.7 mL, 0.2% w/v) was added stepwise to 1.5 mL of chitosan solution (0.2% w/v) under magnetic stirring (700 rpm) at room temperature. In order to load chitosan nanoparticles with DHE, DHE was incubated in the initial CS solution for 4 minutes, followed by the addition of the TPP solution as described above.

CS-NPs were loaded with different concentrations of DHE: 0.5 mg, 0.99 mg, and 1.6 mg (20%, 30%, and 40% theoretical loading). Additionally, different ratios of CS: TPP and loading mechanisms were evaluated. The resulting best fit with 20% DHE loading was utilized for the *in vivo* study.

Nanoparticle solutions were centrifuged in centrifuge tubes at 20,000 × g for 20 min at controlled room temperature (CRT), and then the supernatant was discarded. The resulting nanoparticles were lyophilized until use.

2.3. High-Performance Liquid Chromatography (HPLC) Assay. Analysis of DHE was performed on a Shimadzu reversed phase high-performance Liquid chromatography (RP-HPLC) system (model UV 1601 PC, Kyoto, Japan) equipped with an Agela-Unisol RP-C18 column (4.6 × 150 mm, 5 μm) and Vp 6.14 software. The mobile phase consisted of water, acetonitrile, triethylamine, and trifluoroacetic acid (70 : 30 : 0.1 : 0.1) (pH 2.5) delivered at a flow rate of 1.5 mL/min at room temperature. For aqueous samples, the UV/VIS detector was set at 280 nm, and plasma samples were analyzed with fluorescence detection at Ex 280 nm and Em 350 nm for enhanced sensitivity.

2.4. Characterization of DHE-Loaded Chitosan Nanoparticles

2.4.1. Morphological Examination. A scanning electron microscope (SEM) was used to evaluate the morphology and particle size distribution of the DHE-loaded CS-NPs. Freeze-dried nanoparticles were coated with 2 nm gold at room

temperature. The grid was examined with field emission gum-scanning electron microscopy (FEG-SEM) (FEI QUANTA FEG 450).

Particle size and zeta-potential of CS-NPs were determined using dynamic light scattering using a Zetasizer (Malvern Instruments, Malvern, UK).

2.4.2. Determination of Entrapment Efficiency (EE) and Drug Loading (DL) Percentages. The DHE-loaded nanoparticles were separated from the aqueous medium by ultracentrifugation at 20,000 × g for 20 min at CRT (Beckman centrifuge, Fullerton, Canada). The clear supernatant was diluted in triplicate and analyzed for free nonentrapped DHE by high-performance liquid chromatography-UV detection as described above. The difference between the amount of DHE used for nanoparticle preparation and the free nonentrapped DHE in the supernatant was considered as the amount of DHE loaded into the nanoparticles.

The efficiency of drug encapsulation efficiency (EE) and drug loading (DL) of nanoparticles were calculated according to the following equations:

EE% = [(total weight of DHE added − weight of free nonentrapped DHE)/(total weight of DHE added)] × 100.

DL% = [(total weight of DHE added − weight of free nonentrapped DHE)/[(total weight of DHE added − weight of free nonentrapped DHE + weight of polymer added))] × 100.

2.5. In Vitro Release of DHE from DHE CS-NPs. Sink conditions for the drug release studies were assessed using small portions of nanoparticle suspension containing 1 mg of DHE and diluted in 10 mL of purified water. Samples were then incubated at 37°C and agitated (500 rpm). At predetermined time intervals, 1 mL samples were withdrawn and replaced by fresh phosphate buffer media. The samples were centrifuged (18,000×g for 20 min) and the released DHE was determined by HPLC/UV.

2.6. In Vivo Nasal Bioavailability Studies. The nasal absorption of DHE and DHE-loaded nanoparticles containing 20% DHE were studied *in vivo* using male Sprague–Dawley (SD) rats (Animal House Institute, Jordan University of Science and Technology) weighing 260–350 g ($n = 9$) [27, 28]. This research was approved and monitored by the Institutional Animal Care and Use Committee (IACUC) of Jordan University of Science and Technology prior to its initiation and during its execution.

All surgical procedures were performed under anesthesia (intraperitoneal injection of xylazine 2% (0.1 mL) and ketamine 10% (0.1 mL)). The rat's trachea was cut and separated from the gastrointestinal tract to ensure the full administration of the dose via the nasal cavity.

The intranasal (i.n.) solution and nanoparticle formulations were administered using a microsyringe with an elastic top into the rat's nostril at a dose of 0.5 mg DHE/kg. Rats were given intravenous (i.v.) injections at a dose equal to 0.5 mg DHE/kg rat weight for the determination of absolute bioavailability. Testing for each formulation was done in triplicate as the approval number of the *in vivo* study was 3 rats for intravenous, 3 rats for intranasal solution, and 3 rats for intranasal nanoparticle formulation. The nanoparticle formulation pH was maintained within the pH range of the nasal mucosa (pH 5.5–6.5) to avoid nasal irritation [46].

After administration of i.v. and i.n. DHE, the blood levels were determined by collecting 150 μL blood samples from rat tail veins stimulated by the milking mechanism.

Blood samples were collected at 0, 5, 10, 20, 40, and 60 minutes and then centrifuged at 6,000 rpm for 15 min. Plasma (60 μL) was frozen until the time of analysis.

2.7. Preparation of Plasma Samples by Liquid-Liquid Extraction. Tertiary butyl methyl ether (TBME) (1 mL) was added to 60 μL plasma samples and vortexed for 5 min and then centrifuged at 13,000 rpm for 6 min. After separation, the organic layer was carefully removed and placed in a glass tube. Following the same procedure, another extraction was performed, and the resulting organic layer was also added to the glass tube. 200 μL of phosphoric acid 0.03 M was added to the tube and vortexed for 2 min. In a centrifugal evaporator, the TBME mixture was dried for 4 min under vacuum with no heat. The remaining aqueous phosphoric acid layer was carefully collected into glass inserts and 60 μL aliquots were injected into the HPLC.

2.8. Statistical Analysis. Pairs of groups were compared by Student's t-test. Differences between groups were considered significant at $p < 0.05$. Values for all measurements are expressed as means ± SD.

3. Results

Chitosan nanoparticles were prepared using a modified ionotropic gelation procedure by adding TPP aqueous solution to the chitosan solution. Figure 2 illustrates representative SEM imagies of CS-NPs. Nanoparticles loaded with 20, 30, or 40% DHE were obtained as described above. Characterization of particle size, loading, encapsulation efficiency, polydiversity index, and zeta-potential is illustrated in Table 1. The actual loading of DHE in the nanoparticles was measured and compared to the theoretical loading. The encapsulation efficiency for the 20, 30, or 40% loading was 95%, 83%, and 84%, respectively. The average particle size ranged from 395 to 689 nm (Table 1).

The integration of higher concentrations of DHE led to an increase in the size of the nanoparticles. The results illustrated that the loading capacity and size of the nanoparticles were affected by the DHE concentration in the chitosan solution used to prepare the nanoparticles. These results agree well with the nature of DHE, which is positively charged under acidic conditions, leading to repulsion between positively charged groups in CS and DHE. Thus, the better encapsulation efficiency and smaller nanoparticles loaded with 20% DHE were selected for further *in vivo* testing.

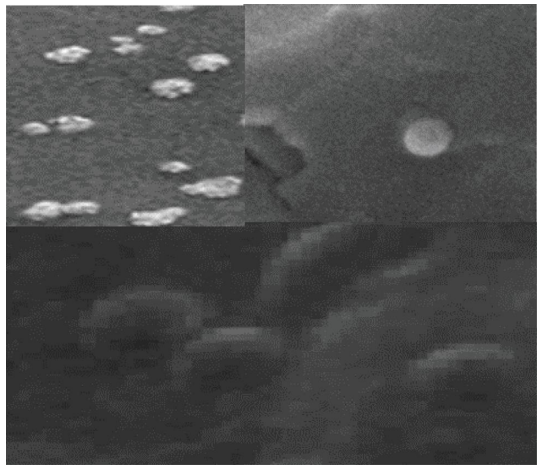

Figure 2: Scanning electron microscope (SEM) images of dihydroergotamine-loaded chitosan nanoparticles.

Table 1: Physicochemical properties of chitosan nanoparticles prepared with different loading of DHE.

DHE theoretical loading in chitosan nanoparticles (%)	Particle size (nm)	Loading (%)	Encapsulation efficiency (%)	Polydiversity index	Zeta-potential (mV)
20	395 ± 59	19 ± 2.6	95 ± 13	0.438 ± 0.031	+26.5 ± 1.9
30	402 ± 65	25 ± 5	83 ± 16.7	0.436 ± 0.007	+30.3 ± 1.3
40	689 ± 67	33.7 ± 6	84 ± 15	0.69 ± 0.164	+35 ± 2.93

The HPLC method was developed and validated with a calibration curve of $R^2 = 0.999949$. The recovery of the area under the curve of aqueous samples and plasma was found to be higher than 90%. Double extraction was used to increase sensitivity and improve DHE detection limits. The limit of detection was about 1.5 ng/ml, while the limit of quantification reached 1.7 ng/ml.

The *in vitro* release profile of DHE chitosan nanoparticles under sink conditions is shown in Figure 3. It shows that about 53% of DHE was released in the dissolution media within 20 minutes and 82% ± 3.2 after 60 minutes. This release profile meshes well with acute migraine treatment strategies.

DHE was administered to rats intravenously at 0.5 mg/kg and i.n. solution and i.n. CS-NPs were also administered at 0.5 mg/kg. The resulting blood plasma concentration of DHE versus time is shown in Figure 4. Intravenous administration resulted in significantly higher initial blood plasma concentrations than i.n. formulations ($p < 0.05$). At ten min following i.v. injection, DHE concentration peaked at 222.2 ng/mL±8 and then declined in a biphasic speed, first rapidly, then tapering off slowly. Absolute bioavailability of DHE compared to i.v. administration was 53.2 ± 7.7% for i.n. solution and 82.5 ± 12.3% for i.n. DHE CS-NPs (Table 2).

DHE was analyzed for pharmacokinetic parameters after i.v. and i.n. administration (Table 3). T_{max} was reached at 40 minutes for both intranasal formulations, with C_{max} of 213 ± 32 ng/mL (i.n. nanoparticles) and 161 ± 10.5 ng/mL (i.n. solution). The i.v. DHE solution reached T_{max} after only 10 minutes with a C_{max} of 483.3 ± 69.9 ng/mL. The half-life ($T_{1/2}$) of DHE in i.v. solution, i.n. nanoparticles, and i.n. solution was 14.0, 16.1, and 20.2 min, respectively.

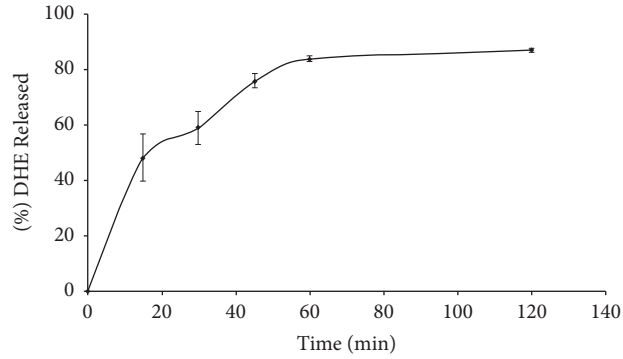

Figure 3: *In vitro* release profile of DHE from DHE-loaded chitosan nanoparticle in dissolution medium. Values are means ± SD ($n = 3$).

The DHE i.v. solution and i.n. nanoparticles had similar elimination rate constants (K_e) (0.049 min^{-1} and 0.043 min^{-1}, respectively), while the i.n. solution differed slightly (0.034 min^{-1}). However, the difference was not statistically significant ($p < 0.05$).

4. Discussion

Due to the demonstrated effectiveness of chitosan as a delivery vehicle [36, 47], DHE was prepared as chitosan nanoparticles using ionotropic gelation of chitosan with TPP anions. Increased loading of DHE in nanoparticles produced a statistically significant ($p < 0.05$) increase in particle size. Therefore, loading additional DHE into chitosan nanoparticles increases the ionic charge ratio relative to chitosan,

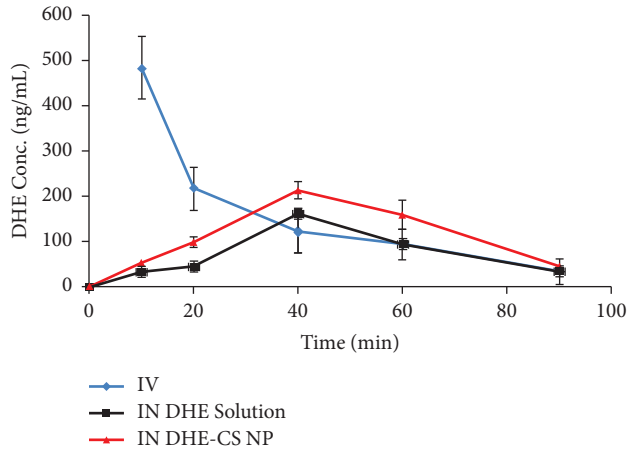

Figure 4: Plasma concentration versus time profile following 0.5 mg/kg intravenous, intranasal administration of DHE solution, and DHE loaded in chitosan nanoparticles (DHE-CS NP). Values are means ± SD ($n = 3$).

Table 2: Area under the curve (AUC) and absolute bioavailability (%) of DHE formulations in rats ($n = 3$).

Route	Dose (mg/kg)	$AUC_{0min-\infty}$ (ng·min·kg/mL/mg) Mean	Absolute bioavailability (%) Mean ± STDV
Intravenous DHE solution	0.4	13299.9 ± 973.8	100
Intranasal DHE solution	0.4	7066.7 ± 1088.3	53.2 ± 7.7
Intranasal DHE chitosan nanoparticles	0.4	10905 ± 1153.9	82.5 ± 12.3

Table 3: Pharmacokinetic parameters following intravenous (i.v.) and intranasal (i.n.) administration of DHE formulations to rats ($n = 3$).

Parameter	Intravenous DHE solution Mean ± STDV	Intranasal DHE nanoparticles Mean ± STDV	Intranasal DHE solution Mean ± STDV
C_{max} (ng/mL)	483.3 ± 69.9	213 ± 32	161 ± 10.5
T_{max} (min)	0	40	40
$T_{1/2}$ (min)	14 ± 39	16.1 ± 3.9	20.2 ± 9.1
K_e (min^{-1})	0.049 ± 0.05	0.043 ± 0.01	0.034 ± 0.01

as has been seen in the literature [47, 48]. The TPP polyanion favors the electrostatic interaction between the oppositely charged components. The final DHE CS-NPs had a zeta-potential of +26.5 mV and a polydiversity index of 0.438, which were in line with other chitosan nanoparticle formulations [49]. A higher zeta-potential indicates stability and a polydiversity index below 0.5 can indicate a similar distribution of particle size.

DHE was released rapidly from chitosan nanoparticles under *in vitro* sink conditions. The hydrophilic nature of chitosan is the most likely variable affecting DHE release from the nanoparticles. Chitosan has been shown to improve the dissolution of poorly water-soluble drugs [50, 51] by allowing an aqueous dissolution medium to access and dissolve the drug. Another factor could be that DHE was loaded into the CS-NPs near the surface. On the other hand, one might suggest that the main reason for this burst release of DHE from nanoparticles is its detachment from the polymer matrix due to repulsion.

Chitosan nanoparticles significantly enhanced *in vivo* DHE systemic absorption after i.n. administration (absolute bioavailability = 82.5 ± 12.3) when compared to i.v. dosing of DHE solution and to i.n. DHE solution ($p < 0.05$), although both i.n. solution and i.n. chitosan nanoparticles were absorbed quite rapidly.

When administering intranasal DHE in rats, limited nostril capacity limits the amount of drug absorbed through the nostrils, with 35%–40% absolute bioavailability [52], which is in the typical range of bioavailability for most intranasal DHE formulations [53]. Thus, chitosan nanoparticles as a delivery system for DHE enhanced absorption, most likely through the demonstrated ability of chitosan to adhere to mucosal tissues [45, 54–59]. However, chitosan's increased surface area [60] and the ability to open tight junctions [61] can also improve absorption. Rapid absorption through the nasal mucosa would be beneficial when attempting to quickly dose an uncooperative patient while avoiding injections, which often leads to patient noncompliance.

The collected data showed that NP preparation has a superior bioavailability enhancement compared to conventional nasal solution preparation. In addition to

chitosan's mucoadhesive properties, its positive charge interacts with the anions on the mucus membrane surface to increase retention time, improving the drug absorption [62, 63].

The pharmacokinetic parameters ultimately demonstrate the concept of absolute bioavailability improvement. DHE CS-NPs' half-life (14.12 min) is higher than the solution (10.86 min), which indicates a longer therapeutic time. Studies have found the half-life of DHE after i.v. injection in beagle dogs to be 40.79 (0.5 mg)–70.13 min (1.0 mg) [64] and 104.42 minutes (2.0 mg i.v. injection) [65]. The pharmacokinetics of DHE after i.n. and i.v. administration in rats was analyzed. Chitosan nanoparticles demonstrated potential for intranasal administration of DHE due to increased absorption compared to that of i.n. solution. Although both formulations reached maximum concentration within the same timeframe, CS-NPs followed a pattern of greater release. Rapid drug absorption is of particular benefit in migraines where patients require rapid relief or when a dose is missed. Furthermore, the preparation of DHE from chitosan nanoparticles appears to have overcome the slow onset of action which has been demonstrated with DHE [66]. Due to the sensitivity of the nasal mucosa, prophylactic nasal administration of DHE should be used with caution. Further clinical evaluation is warranted to determine the optimal dosing strategy of DHE CS-NPs for rapid relief of migraine.

5. Conclusion

Formulating DHE into chitosan nanoparticles has the potential to improve the systemic absorption of the drug by around 55%, resulting in a more rapid onset of action with a greater systemic bioavailability over DHE intranasal solution. This formulation could benefit migraine patients with improved pain relief, but it would need further clinical evaluation to illustrate its value.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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