ejpmr, 2018,5(3), 349-354

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SPHERICAL CRYSTALS OF METRONIDAZOLE: TO IMPROVE SOLUBILITY, DISSOLUTION RATE AND MICROMERITIC PROPERTIES

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Article Received on 05/01/2018 **Article Revised on 08/01/2018** **Article Accepted on 18/02/2018**

ABSTRACT

Spherical crystals of Metronidazole were prepared by Quasi Emulsion Solvent Diffusion method using a three solvent system comprising ethyl acetate as a good solvent, cyclohexane as a poor solvent and water as a bridging liquid. Hydroxy propyl methyl cellulose (HPMC) was used as hydrophilic polymer. The obtained spherical agglomerates were characterised by various parameters such as melting point, optical microscopy, particle size, angle of repose, crystal density, porosity, scanning electron microscopy, infra red spectroscopy, powder x-ray diffraction studies, solubility studies, *in-vitro* release studies. The prepared agglomerates showed marked increase in solubility and its dissolution behaviour than the pure one, and found improved micromeritics properties. It was revealed that the above spherical agglomerates exhibited considerable improvement in flowability, packability, wettability and compressibility.

KEYWORDS: Metronidazole, Spherical agglomerates, Quasi emulsion solvent diffusion, Dissolution, Flowability, Packability.

INTRODUCTION

Spherical crystallization is the novel agglomeration technique that can directly transfer the fine crystals produced in the crystallization in to a spherical shape. It is a particle engineering technique by which crystallization and agglomeration can be carried out simultaneously in one step to transfer crystals directly into a compacted spherical form.[1]

This technique involves the formation of agglomerated crystals which could be easily compounded with other pharmaceutical ingredients due to their spherical shape using this technology. Physicochemical properties of pharmaceutical crystals are dramatically improved for pharmaceutical processing like milling, mixing and tabletting because of their excellent flowability and packability.[2] Spherical crystals can be prepared by various methods such as simple spherical crystallization, emulsion solvent diffusion, ammonia diffusion and neutralization method. These crystallization techniques had already been successfully applied for several drugs to improve their micro meritic properties such as flowability, packability, density, particle size, shape, solubility and percentage drug release.[3-8]

In this present study, it was planned to prepare the spherical agglomerates of metronidazole (Fig 1) and to investigate the characterization of obtained crystals using various evaluation parameters. Since, it was also planned to differentiate the prepared agglomerates from pure form with their solubility measurement and *in-vitro* drug release profile.

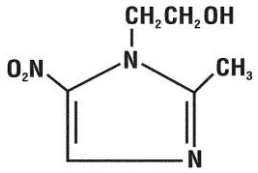

Fig 1: Chemical structure for metronidazole.

MATERIAL AND METHODS

Materials

Metronidazole was procured from Triveni Interchem Pvt Ltd. Gujarat, India. The solvents used for crystallization were ethyl acetate, cyclohexane and methanol and obtained from Merck Pvt. Ltd. Mumbai, India. All other chemicals used were of analytical grade.

Preparation of spherical agglomerates by quasi emulsion solvent diffusion method

Ethyl acetate was taken as a good solvent, cyclohexane and water were taken as poor solvent and bridging liquid respectively. About 1 gm of pure metronidazole powder was weighed and taken in a beaker, 60 ml of ethyl acetate was added to it and warmed in a water bath to dissolve the HPMC. About 60 ml of cyclohexane was taken in a beaker and placed in the cold water bath which was on the magnetic stirrer. The magnetic stirrer was maintained at 300-350 RPM. The ethyl acetate solution

was poured slowly into cyclohexane. Then 40 ml of HPMC solution was added into the mixture drop by drop. Initially, the salted drug deposited on the bottom of the beaker, on further adding of HPMC solution the emulsion was formed. Then the emulsion was left standing for 20 minutes. Then the mixture was filtered with whatmann filter paper and dried at room temperature. The dried crystals are collected and kept in the desiccator.

Characterization of Spherical Agglomerates

Melting point

The determination of melting point of the spherical crystals was carried out in open capillary method using electrical melting point apparatus (Remi Instruments).

Optical Microscopy

The pure drug and prepared spherical agglomerates were viewed under optical microscope for their physical characterization. The samples were prepared by placing a small amount of drug and spherical agglomerates on the slide, dispersed in a drop of liquid paraffin and covered with a cover slip. The slides were visualized by means of binocular polarizing microscope under 10X/0.25 and 40X/0.44. Photomicrographs were taken by using cannon digital camera.

Determination of Particle Size[9]

Particle size was determined by using optical microscope. The ocular micrometer was first calibrated by using the stage micrometer in which the sample of particle size to be determined was sprinkled on glass slide. The cover slip was carefully placed over the slide and the sizes of particles are measured with the help of ocular micrometer. At least a minimum of 100 particles were measured in various sizes and average was calculated.

Angle of Repose

Angle of Repose of drug powder and agglomerates were assessed using fixed funnel method. The funnel was fixed at a particular height on a stand. The sample was passed slowly through the funnel until it forms a pile. Further addition of drug was stopped as soon as the pile touches the tip of the funnel. The radius "r" and height "h" was measured and the angle of repose was calculated by using the formula,

$\text{Tan}(\theta) = h/r$

Where, h = height of the pile

r = radius of the pile

(θ) = Angle of Repose

Table 1: Relationship between Angle of the Repose and Flowability

S.No	Angle of Repose	Flowability
1	<20	Excellent
2	20-30	Good
3	30-40	Passable
4	>40	Very Poor

Determination of Density

Crystal Density

True density of sample was determined by liquid (water) displacement method. Weight of the empty flask (a) and weight of the flask along with liquid (b) were determined accurately. An amount of sample powder was replaced into the flask and weight was measured (c) accurately. Liquid was poured in to flask until drug powder and liquid together occupied the full volume of the flask and weight of the flask was measured again. True density was calculated by.

$$True\ density = \frac{weight\ of\ sample\ (c-a)}{true\ volume\ of\ sample\ (d-b)}$$

Bulk Density and porosity

An amount (2 g) of sample was transferred into a measuring cylinder and volume (bulk volume) was measured in cm^3.

$$Bulk\ density = \frac{weight\ of\ sample}{bulk\ volume\ of\ sample}$$

$$Porosity\ (\varepsilon) = \frac{bulk\ volume - true\ volume}{bulk\ volume\ of\ drug}$$

Scanning Electron Microscopy (SEM)[10]

The surface morphology of the agglomerates and pure drug were assessed by scanning electron microscope.

FT-IR Spectroscopy[11]

Infra red spectra of crystal forms and pure drug were scanned on (Shimadzu, England) using KBr pellets. Samples (2 - 2.5 mg) were triturated with dried potassium bromide (100 mg) using glass mortar and pestle. The mixture after a grinding into fine powder was compressed into a pellet form by applying a pressure of about 10 kg/cm for 3 minutes using hydraulic press. The resultant pellet was mounted in a suitable holder in the FT-IR spectrometer and full range spectra of samples were recorded from 4000 cm^{-1} to 400 cm^{-1}.

Powder X-ray Diffraction Studies (XRD)[12]

A Powder XRD was used to identify the polymorphs. The samples were exposed to Cu Ka radiation (45 kV and 40 mA) and were scanned from 2˚ to 50 ˚2 theta at step size of 0.01˚ and 3 sec/step. The divergent slit size was 0.9570˚, the receiving slit 1 mm, and detector slit 0.1mm.Data were collected by a solid – state (SiLi) detector. Data was analyzed using DMax-3 software. Specimen was packed in a specimen holder made of aluminum.

The powders were passed through a 100 mesh sieve and were placed into the sample holder by side drift technique. The holder consisted of central cavity. In order to prepare a sample for analysis, a glass slide was clipped up to the top face of sample holder so as to form a wall. Each powder was filled into the holder, gently and used for XRD analysis.

Solubility Studies

Solubility studies of pure drug and spherical agglomerates of Metronidazole were carried out by using 0.1N HCl. Saturated solution was made by adding excess drug to the medium and shaking on the shaker for 4 h at 25 ± 0.5˚C under constant stirring. After this procedure the solutions were filtered, diluted and analyzed by UV spectrophotometer. Three determinations were carried out for each sample to calculate the solubility of Metronidazole.

***In-vitro* Drug Release Studies**

The dissolution test was performed using United States Pharmacopoeia (USP) type II (paddle) apparatus with 900 ml of 0.1N HCl at 37± 0.5˚C and at 100 rpm. The dissolution was carried out for 3 hours. Samples (1ml) were withdrawn at 30 minutes interval. The same volume of medium was replaced immediately to maintain the sink condition. The absorbance of the resulting solutions (after filtering through Whatmann filter paper) was taken at 289 nm after suitable dilutions using 0.1 N HCl.

Micromeritic Properties

Flow property increase with the decrease in angle of repose. In this study, angle of repose of pure drug was measured as 37°, where as Spherical crystal was 28°, hence the flow property was found good in agglomerates. This may be due to the formation of smaller, uniform shaped crystals. The loose bulk density (LBD) of pure drug was found to be 0.184gm/ml, and Spherical crystal was 0.247gm/ml. The Tapped density (TBD) of pure drug was 0.278gm/ml, and Spherical crystal was 0.276gm/ml. The particle size of pure drug of metronidazole was 87µ, where as Spherical a crystal was 131µ.. It is quite evident from the table (Table 2) that the spherical agglomerates showed excellent compressibility which is indicated by lower carr's index value (for pure drug, it was 33.57%, where as for spherical crystals 10.5%) and also showed excellent Hausner's ratio. The comparative low value of angle of repose, Carr's index and Hausner's ratio of metronidazole agglomerates indicated their good flowability, packability and compressibility.

RESULT AND DISCUSSION

Table 2 : Micromeritic properties of powder and spherical crystals of Metronidazole.

Parameter	LBD	TBD	Carr's Index (%)	Hausners's Ratio	Angle of Repose	Particle size (µ)
Drug	0.184 0.00058	0.278 0.001	33.57 0.0058	1.5 0.0058	37 .34 0.27	87.09 0.12
Spherical Crystal	0.247 0.00057	0.276 0.00058	10.5 0.00058	1.11 0.0058	28 .49 0.015	131.82 0.22

Scanning Electron Microscope

The pure drug shows rod and irregular shaped crystals. The surface morphology of the prepared agglomerates shows that they were spherical shape. It will give good flow and compression properties.

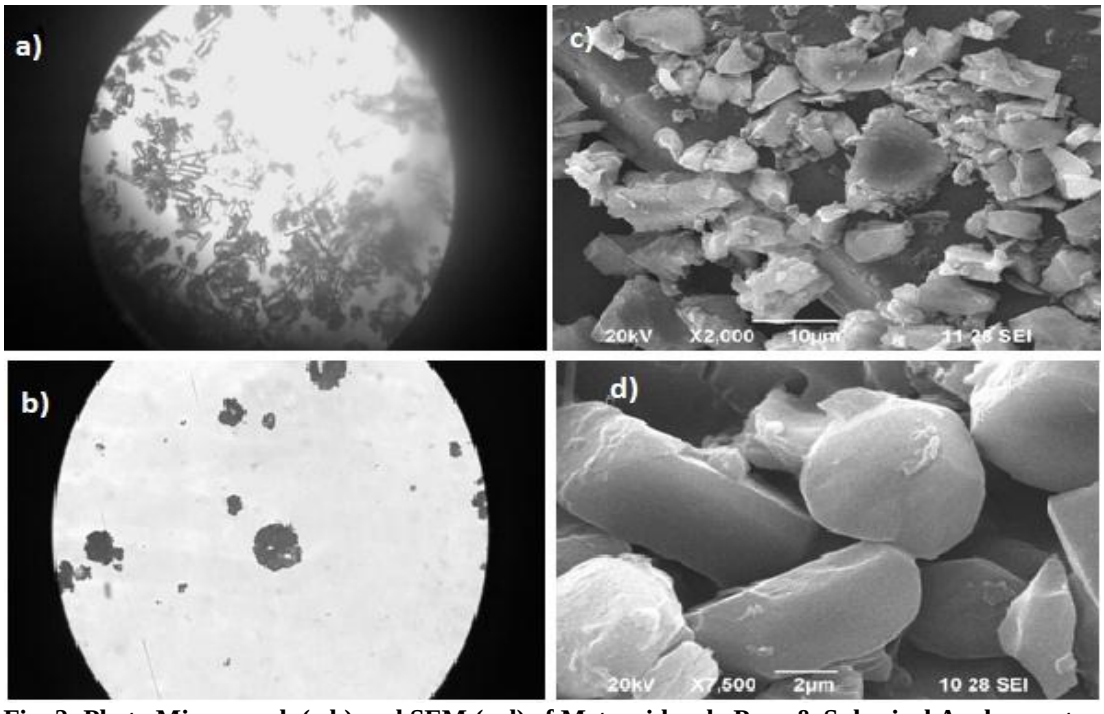

Fig. 2: Photo Micrograph (a,b) and SEM (c,d) of Metronidazole Pure & Spherical Agglomerates.

FT-IR Spectroscopy

From IR spectrum of amorphous and spherical crystals, it revealed that there was no alternation in the characteristic peaks of functional groups (shown in Fig 3), which indicated that there was no interaction between the drug and solvents during the crystallization process.

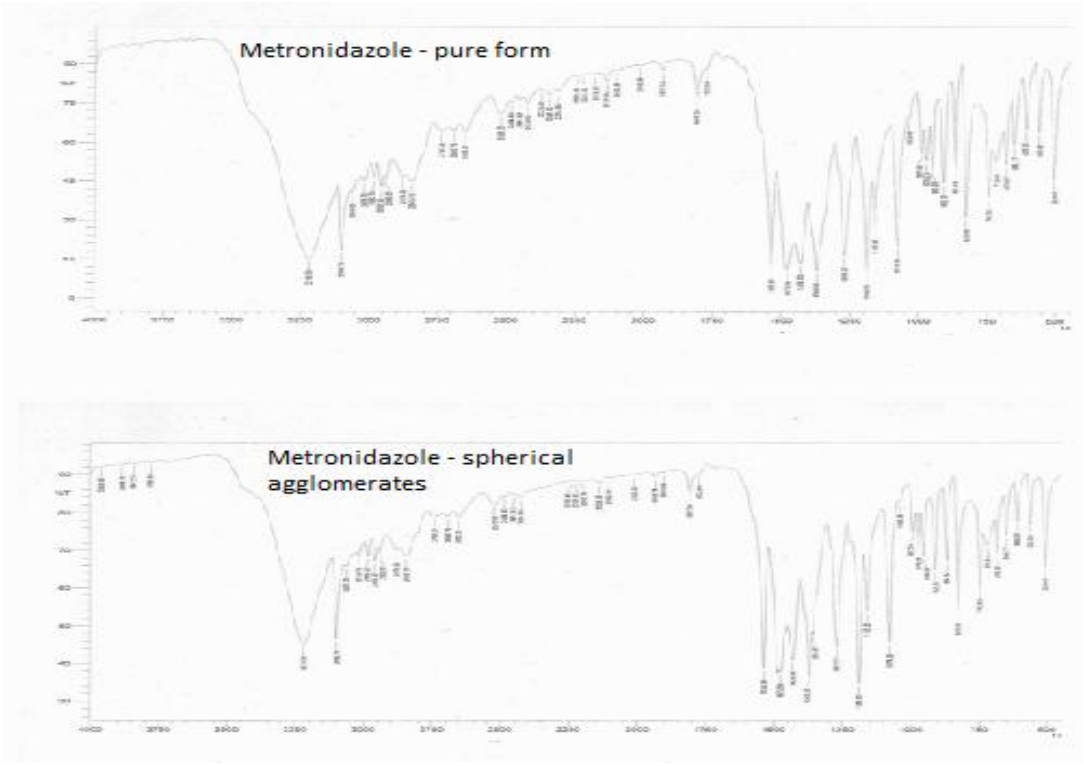

Fig. 3: FT-IR spectrum for Metronidazole pure drug & Spherical Crystal.

Powder XRD Analysis

The difference in the position of 2θ values of spherical crystals forms of metronidazole were confirmed by well resolved diffraction patterns with their corresponding peaks which was displayed on table. Appearance and disappearance of some peaks on the XRD spectrum (Fig. 4 and Table 3) was clearly indicating the formation of different crystal lattice.

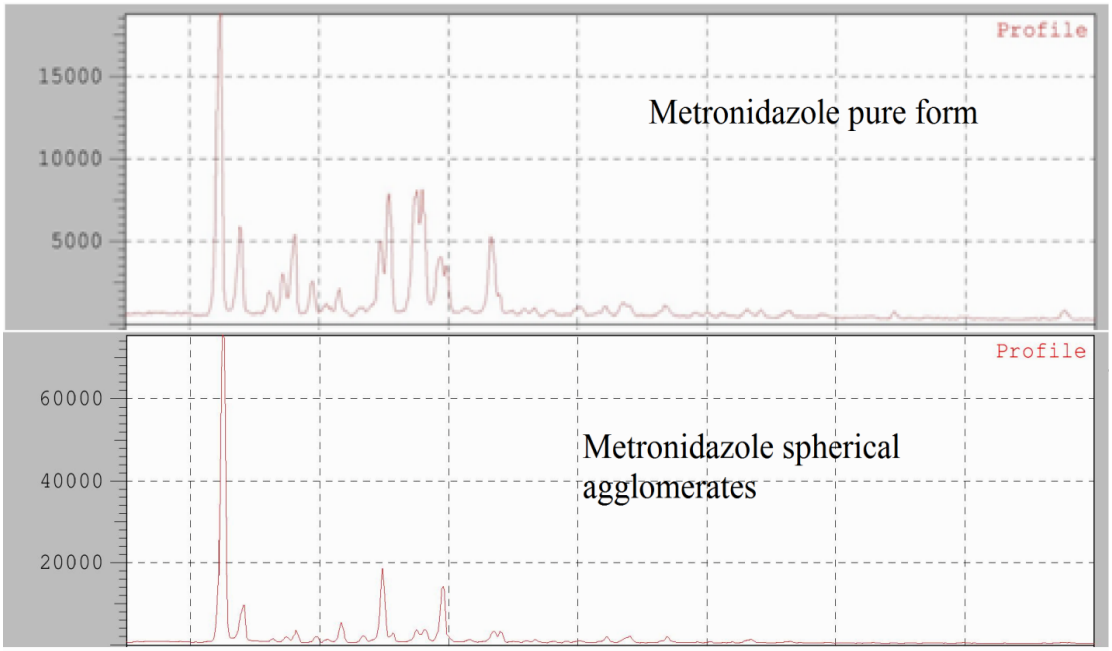

Fig. 4: Powder XRD graph for pure and spherical crystal.

Table 3: Powder XRD study data.

Pure Drug		Spherical Agglomerates	
°2 theta	Intensity (CPS)	°2 theta	Intensity (CPS)
12.51	**25599**	**12.30**	**7266**
14.03	3674	13.90	2009
18.18	1040	16.21	521
21.69	1904	17.25	908
24.88	**6987**	18.06	1913
25.62	885	19.48	797
27.50	1188	20.58	246
28.20	1214	21.54	587
29.54	**5693**	24.70	1732
33.51	1064	**25.38**	**2910**
34.10	1030	**27.50**	**3004**

Dissolution Studies

The solubility study showed that spherical crystals have good solubility in water as well as in other solvents. The pure drug solubility was 25.56mg/ml, where as spherical agglomerate was 29.83mg/ml which was determined by measuring the absorbance of the solution at 289 nm.

The absorbance for the different concentration (1-10 µg/ml) was recorded at above said wavelength to construct the standard curve and the regression equation of the calibration curve was found to be y=0.0327x+0.0088 which was shown in (Fig. 5).

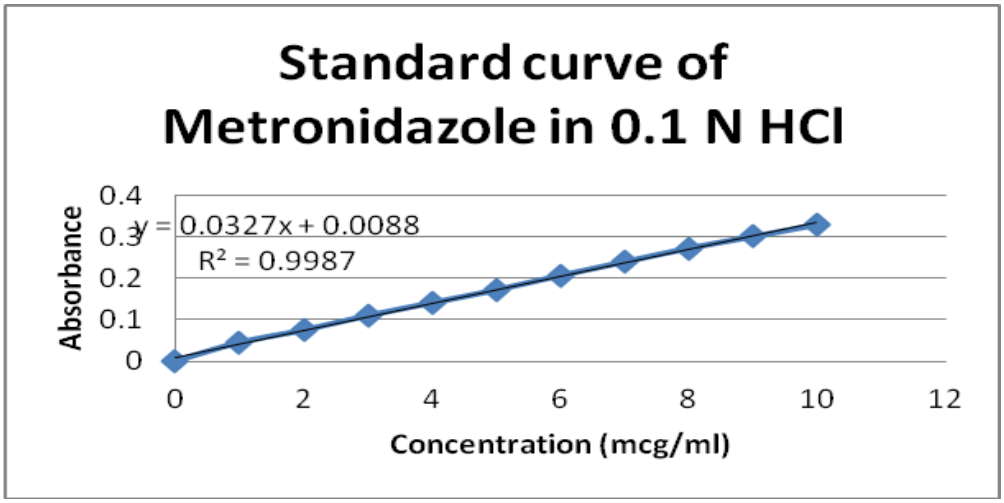

Fig.5: Calibration curve for Metronidazole (λmax at 289nm).

The *in-vitro* dissolution study of amorphous and crystalline forms were performed using 0.1N HCl and cumulative percentage drug release of spherical crystals was calculated by the use of standard calibration graph obtained at 289nm. The results of in vitro dissolution studies are shown in (**Fig. 6**). Pure Metronidazole exhibited least dissolution pattern compared to spherical crystal at 180 minutes only 85.5 % of drug goes into solution while at same time it was 95.5 %.

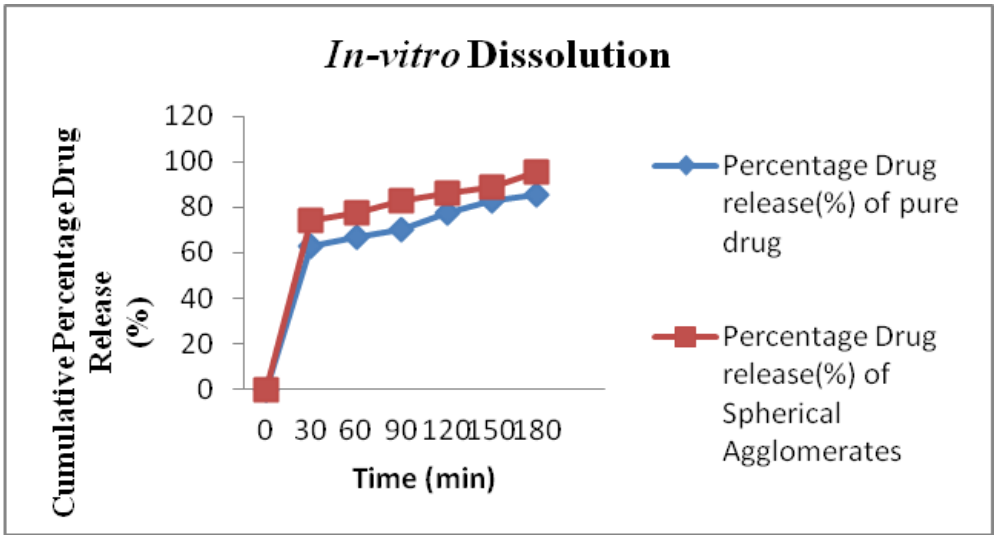

Fig.6: Graph for comparative dissolution study data.

CONCLUSION

The spherical crystallization employs three solvents, ethyl acetate, cyclo hexane and water. The ethyl acetate is good solvent for soluble the metronidazole, Where as cyclo hexane act as poor solvent and water is a bridging liquid. This process is carried out under the controlled physical factors such as agitation, temperature chemical factors such as solubility, raw material concentration and solvent quantity. Metronidazole is a drug having a specific solubility profile, so for the preparation of spherical agglomerates Quasi Emulsion solvent diffusion method was used. Infrared spectroscopic studies, Scanning Electron microscopy, X-ray diffraction method were used for characterization of pure drug and its agglomerates. IR spectroscopy reveals that there are no chemical changes when comparing the pure drug and spherical agglomerates. SEM studies revealed that the spherical agglomerates possess a good spherical shape. Hence it is revealed that micromeritics properties, solubility and *in-vitro* dissolution rate is increased when compared to the pure metronidazole.

ACKNOWLEDGEMENT

The authors are grateful to the authorities of The Erode College of Pharmacy, Erode, Tamilnadu, for providing the facilities to complete our work successfully.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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