Formulation Development and Evaluation of Microemulsion Based Lornoxicam Gel

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The objective of the existing research was formulation development and preparation of microemulsion loaded emulgel in augmenting the topical delivery of Lornoxicam. Emulsion and gel combined are recognized as Emulgels. Gelling agents Carbopol 940, 974, 980 were used to formulating Lornoxicam emulgel. The drug release from emulgel was determined depending on the different gelling agents. Clove oil for internal phase and Polyethylene glycol 200 was applied as co-surfactant and Tween 20 as permeation enhancer. Prepared emulgels were evaluated for in-vitro and in-vivo anti-inflammatory activity using Albino mice and stability studies. The optimized batch of emulgel gave better results when compared with the marketed Diclofenac sodium gel. 90.42% drug release was detected in 8 hr. Prepared formulation was free from skin irritation and detected with 62.02% inhibition of edema. Correspondingly, prepared formulations were found to be stable. Developed microemulsion increased solubility of drugs, so that less soluble drugs can be applied in the formulation. Carbopol 980 (1.5 %) gave better results for emulgel. Therefore, microemulsion-loaded Lornoxicam Emulgel detected with better analgesic effect.

Keywords: Carbopol 980; Clove oil, Emulgels; Lornoxicam; Microemulsion; PEG 200.

For the localized effect of drugs, topical drug delivery systems are preferred. Topical drug delivery systems used for dermal and transdermal diseases. The advantage of the topical route is formulation is directly applied to the skin and increases the permeability of drugs1. By using topical drug delivery systems disadvantages of an oral route can be reduced. A targeted drug delivery system increases patient compliance. Inflammation is a local protective response of the body tissue injury2,3. An emulsion is a heterogeneous system that contains one liquid in another which is not miscible, prepared a coarse dispersion, and stabilize by adding emulsifying agent Hoar and Schulman introduced the microemulsion. Isotropic mixtures of oil, water, and surfactant and sometimes co-surfactant known as Microemulsion. Microemulsions are thermodynamically stable. Droplet size is >0.15 micron and solution is transparent. Microemulsions require a higher amount of surfactant, in the range of 6-8% by
total weight contrasting with a value of 2-3% for emulsions. Phase diagram used for obtaining the microemulsion region. J. Willard Gibbs proposed the phase diagram rules. Titration method used for phase diagram and External phase titrated with internal phase. Cloudiness is obtained at the end of the titration. Lastly, the coarse emulsion will be prepared and titrated by a co-surfactant till a transparent solution is obtained. The co-surfactant shows the microemulsion region in a different ratio which is stable, called as “pseudo-component”4-7.

A liquid or semi-liquid thick, clear substance known as Gel. Gel and emulsion combined are called as Emulgels. A gelling agent is an important agent to prepare emulgel. Lornoxicam is a BCS class-2 drug, contains low solubility and high permeability. And in the oral route, it shows the disadvantages like gastric irritation, first-pass metabolism. So that, here is used topical drug delivery system for Lornoxicam by using microemulsion system. Microemulsion increases the solubility and also improves the permeability of the drug8-11.

MATERIALS AND METHODS

Lornoxicam was supplied by Glenmark Pharma. Ltd. Sinner (MH, India), Clove oil, Polyethylene glycol 200 supplied by Thomas Baker, Tween 20 supplied by Thomas Baker, Mumbai (MH, India), Carbopol 940 supplied by Lobie Chemical Mumbai (MH, India), Carbopol 974 and Carbopol 980 was supplied by Lubrizol Pvt. Limited Mumbai (MH, India). Methyl paraben, propyl paraben, Triethanolamine was supplied by Thomas Baker, Mumbai.

Drug and Excipients compatibility studies

Drug and excipients characterization was started with the determination of melting point by using Thiele’s tube method. The drug was filled in capillary at the closed side and capillary kept in liquid paraffin Thiele’s tube. And temperature recorded at which drug melts. Further drug spectra were determined by using UV-visible spectrophotometer12. The maximum wavelength of Lornoxicam was analyzed between 400-200nm. The standard calibration curve for the Lornoxicam stock solution was prepared by 10 mg of Lornoxicam dissolved in 100ml methanol. The 2, 4, 6, 8, 10 ppm concentrations were prepared and diluted by stock solution. 379 nm used as maximum wavelength to obtain absorbance using methanol as blank. The FT-IR spectra of Lornoxicam, clove oil, Tween 20 were recorded using FTIR (SHIMADZU). 4000-400 cm-1 range of frequency was scanned12.

Solubility determination of Lornoxicam

Solubility determination was performed by using the shake flask method. Solubility was obtained for oils, surfactants and co-surfactant. 3 ml solvent was taken in the test tube with saturated drug and the mixture was kept in a cyclometer for 10 min. for mixing purposes. Kept for 72 hrs. In an isothermal shaker at 37 ± 1c. At 5000 rpm samples were centrifuged 15 min and filter through membrane filtration (0.45 micrometer). The supernatant of samples were examined by using UV13,14.

Preparation of Microemulsion

Tween 20, clove oil, PEG 200 were utilized for formulation depending on the maximum solubility of the drug. The titration method was used for the pseudo-ternary phase diagram. 1:1, 1:2, 1:3, 2:1, and 3:1 different ratio taken for surfactant and co-surfactant. 90:10, 80:20, 70:30, and 60:40 ratio taken for oil and sample mixture and Water added slowly and mixed by vortex mixture. Cloudiness was observed at the end point. Surfactant and co-surfactant ratio plotted as a triangle which shows in the dotted region. The pseudo ternary phase diagram gives different region for the preparation of microemulsion. Lornoxicam added oil, surfactant and co-surfactant mixture in different percentages given in table 2 and slowly add water with continuous stirring. At constant stirring and temperature, microemulsion was prepared12,15.

Characterization of prepared microemulsion

Dilution Test and Dye Test

On addition of water in microemulsion, O/W found to be stable whereas W/O detect with breaking of microemulsion. In the dye test, suddan red added in microemulsion and observed on a microscope. The globule observed red and ground was detected as colorless which confirmed developed microemulsion as O/W2.

Measurement of Globule size, PDI and Zeta Potential

Zetasizer Nano-ZS (Malvern Instruments, UK) was used for the determination of globule
size. 1 ml of microemulsion was diluted with doubled distilled water and by zeta cell globule size was obtained. For stability zeta potential was determined. The measurement was performed at 25°C²2,10.

**Measurement Viscosity, Phase Separation and pH determination**

Brookfield Viscometer (DV-E viscometer LV) was applied for measurement of the viscosity by using spindle S-18. Sample were taken in the beaker and the spindle were placed in the beaker containing sample. For the phase separation study, microemulsion centrifuge in centrifugation (Remi Motor, Mumbai) at 5000 rpm for 30 min. By using digital pH meter the pH of the prepared microemulsion was determined and calibrated by using phosphate buffer. All readings was determined in triplicate and average of triplicate was taken for more accuracy16,17.

**Stability Studies**

Accelerated stability study was done for prepared batches at temperature 40 ±2°C and 75±5% RH for 3 months. 0, 30, 60, and 90days at this time points sample taken and examined for any physical changes16.

**Method of preparation of Emulgel**

Four microemulsions formulations were prepared and composition having S/Cos ratio 2:1 was taken for the preparation of gels, Carbopol 940, Carbopol 974 and Carbopol 980 three galling agent was used in different concentrations (0.5-2 %). Microemulsion loaded gel was prepared by using microemulsion as base. In one beaker PEG 200 was taken and preservatives added in that to make oily phase and drug added in this phase. The water was added in Tween 20 to prepare aqueous phase. The oil phase added in aqueous phase with continuous stirring to prepare clear microemulsion. Carbopol 980 was dispersed in distilled water for 24 hr. In 6-6.5 pH was adjusted by using triethanolamine. Gelling phase was added in microemulsion in 1:1 ratio with continuous stirring to obtain transparent microemulsion based gel¹⁸-²⁰.

**Characterization of microemulsion based Gel**

**Physical Appearance and determination of pH**

The color, homogeneity, consistency was determined. By Digital pH meter pH of gel was obtained. 1ml of gel diluted with 9ml of distilled water and readings were taken²¹.

**Rheological study**

Rheological study was performed for the determinations of formulations rheological characteristics. Viscosity is the important evaluation parameter for the topical dosage form. Therefore, viscosities of formulations were determined by using Brookfield viscometer at 37°C by using Spindle number S-64²²-²³.

**Spreadability**

The spreadability apparatus was used for the test. The two glass slides (10 × 10 cm) were placed in this apparatus. 0.5gm sample was kept in glass slides. 100 gm weight kept on upper slide and measure the diameter or length which was of pre-marked circle. The time required to spread the sample was recorded²⁴. Formula-

\[
S=MxL/T
\]

Where,

\[ M= \text{ weight} \]
\[ L= \text{length or diameter} \]
\[ T= \text{time} \]

**Extrudability determination**

The extrudability test was determined to study how much pressure or force is required to expel material from tubes. The gel was extruded from aluminum collapsible tube by applying weights and measured the area for calculation²¹. The formula:

\[
\text{Extrudability} = \frac{\text{Weight (gm)}}{\text{Area (cm}^2\text{)}}
\]

**Globule size and its distribution in emulgel**

Malvern Zetasizer instrument was used to determine the globule size. For obtained clear solution sample was mixed in water. The homogeneous dispersion of gel sample was then placed for the analysis and readings were recorded²⁵,²⁶.

**Stability Studies**

At 40°C and 75% RH samples were kept for 3 months. Any physical/chemical changes in the formulation was studied at the end of study²⁷.

**In-vitro diffusion study**

Modified Franz diffusion cell was used. A glass cylinder used with 10 cm height for diffusion cell. In receptor chamber the phosphate buffer pH 6.8 was kept and in donar receptor gel was applied. The cell was kept in contact with receptor chamber
at 32±1°C. The samples were examined at different time points and analyzed at 276 nm on UV28,29.

**Skin irritation test**
0.5 gm of gel sample applied on skin 1" x 1" (2, 54 x 2, 54 cm) square. The mice were used for skin irritation test. After a 24 hour, the animals were examined for presence of any erythema or swelling.26.

**Anti-inflammatory study**
For anti-inflammatory study albino mice were used. 15 mice were weighed and maintained them on fasting overnight. Control, standard and test 3 group were prepared and in each group 5 animals were taken. 1% (w/v) carrageenan was injected on left hind paw to induce edema. 0.5 gm of gel applied on the skin. At 0, 30, 60, 120 and 180 minute thickness of paw was measured by using vernier caliper. Microemulsion loaded gel and marketed Diclofenac gel were compared with carrageenan. By using ANOVA test results were calculated.29.

**RESULTS AND DISCUSSION**

**Drug and Excipients compatibility studies**
The drug maximum wavelength was checked with UV-Spectrophotometer and characteristic sharp peak were obtained at the 376 nm in phosphate buffer 6.8 solution and 379 nm in methanol solution. 400-200 nm maximum wavelength was analyzed for the samples. 2, 4, 6, 8, 10 ppm concentrations were prepared and diluted with methanol. In the both solution, linearity was detected as well as obtained spectra was matched with the reported spectra of Lornoxicam.30. The FT-IR characterization of Lornoxicam was also recorded and C-O, C-C, N-H, C-H, C=O functional groups were identified stretching was recorded and its matches with the previously reported data. FT-IR spectra also determined for the clove oil and tween 20 excipients and both are found same as reported one.31,32.

**Solubility study of Lornoxicam**
The oil applied as most important excipients in microemulsion. Solubility of all

![Fig. 1. A) Calibration curve of Lornoxicam B) FT-IR spectra of Lornoxicam C) FTIR spectra of clove oil D) FTIR spectra of tween 20](image-url)
different oils shown in below table. The clove oil showed higher solubility as compared to other oils. Solubility study of different surfactant was mentioned in Table 1. Tween 20 showed higher solubility. Highest solubility of drug was found in PEG 200 and hence it was selected as co-surfactant for further study. Table 1 summarized the solubility study of the Lornoxicam drug.

**Preparation of Microemulsion**

From pseudo-ternary phase diagram, Tween 20 as surfactant, Polyethylene glycol taken as co-surfactant and clove oil was taken as oily phase. Microemulsion formation area was found to be greater with and decreased in surfactant & Co-surfactant ratio at 2:1. The ratio 2:1 of surfactant : co-surfactant containing tween 20 and PEG 200 showed more microemulsion region with stability. Table 2 showed the composition of microemulsion formulation.

**Characterization of Microemulsion**

**Dilution Test and Dye Test**

The dilution test is based on the solubility of an external phase of microemulsion. O/W microemulsion can be diluted with water. After dilution of microemulsion with water there is no phase separation indicates that prepared microemulsions were o/w type. Dye test was performed as mentioned in the methods section. The scattered globules appear red with colorless continuous phase and O/W type of emulsion was detected. Figure 2 showed the scattered globules with red color.

**Globule size, Zeta Potential & polydispersibility index**

As per Figure 3 A, a globule size was obtained at 217 nm. Zeta potential is important for stability of formulations and the standard value is

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**Table 1.** Lornoxicam drug solubility (mg/ml) in oil, co-surfactant, surfactant

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oils</th>
<th>Solubility</th>
<th>Surfactant</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Peppermint Oil</td>
<td>0.88</td>
<td>Tween20</td>
<td>3.39</td>
</tr>
<tr>
<td>2</td>
<td>Olive Oil</td>
<td>1.02</td>
<td>Tween80</td>
<td>1.71</td>
</tr>
<tr>
<td>3</td>
<td>Cinnamon Oil</td>
<td>1.83</td>
<td>Span20</td>
<td>1.12</td>
</tr>
<tr>
<td>4</td>
<td>Clove Oil</td>
<td>6.39</td>
<td>Span80</td>
<td>1.43</td>
</tr>
<tr>
<td>5</td>
<td>Eucalyptus Oil</td>
<td>0.41</td>
<td>Transcutol</td>
<td>1.33</td>
</tr>
<tr>
<td>6</td>
<td>Labrafil M 2125</td>
<td>1.60</td>
<td>PEG400</td>
<td>3.36</td>
</tr>
<tr>
<td>7</td>
<td>Labrafil M 2125</td>
<td>1.08</td>
<td>PEG200</td>
<td>4.811</td>
</tr>
<tr>
<td>8</td>
<td>Soybean Oil</td>
<td>1.94</td>
<td>Ethanol</td>
<td>0.56</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>Propylene Glycol</td>
<td>1.02</td>
</tr>
</tbody>
</table>

**Table 2.** Composition of microemulsion formulations

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredients (%w/w)</th>
<th>ME1</th>
<th>ME2</th>
<th>ME3</th>
<th>ME4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lornoxicam</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>Clove oil</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Tween 20</td>
<td>35</td>
<td>33</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>PEG 200</td>
<td>15</td>
<td>17</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>30</td>
<td>30</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>Triethanolamine</td>
<td>Q.S.</td>
<td>Q.S.</td>
<td>Q.S.</td>
<td>Q.S.</td>
</tr>
</tbody>
</table>
up to -30mv. In current formulation, -22mv zeta potential (Figure 3 B) was obtained which indicated the formulation with stability. Polydispersibility index measure the heterogeneity or homogeneity of samples. Standard value of PDI is 0.0 to 1.0. The obtained PDI value for the formulation was 0.300 showed that formulation is stable and particles are uniformly dispersed in system.

**Measurement of Viscosity, Phase separation and pH determination**

Viscosity is resistance to flow, which is an important physicochemical property for topical

| Table 3. Formulation batches of Emulgels (% Weight by weight) |
|-----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Sr. No. | Component     | F1   | F2   | F3   | F4   | F5   | F6   | F7   |
| 1     | Lornoxicam    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
| 2     | Carbopol 940  | 1.5  | 2.0  | -    | -    | -    | -    | -    |
| 3     | Carbopol 974  | -    | -    | 1.5  | 1.75 | 2.0  | -    | -    |
| 4     | Carbopol 980  | -    | -    | -    | -    | -    | 1.5  | 1.75 |
| 5     | Clove oil     | 20   | 20   | 20   | 20   | 20   | 20   | 20   |
| 6     | Tween 20      | 33.3 | 35   | 33.3 | 35   | 33.3 | 35   | 33.3 |
| 7     | PEG 200       | 16.6 | 15   | 16.6 | 15   | 16.6 | 16.6 | 16.6 |
| 8     | Propyl Paraben| 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| 9     | Methyl Paraben| 0.3  | 0.3  | 0.3  | 0.3  | 0.3  | 0.3  | 0.3  |

F= Formulation

| Table 4. Microemulsion based emulgels pH |
|-----------------------------|-------|
| Sr. No. | Formulations | pH value    |
| 1     | F1           | 6.51±0.03  |
| 2     | F2           | 6.44±0.03  |
| 3     | F3           | 6.35±0.05  |
| 4     | F4           | 6.54±0.03  |
| 5     | F5           | 6.36±0.01  |
| 6     | F6           | 6.50±0.01  |
| 7     | F7           | 6.22±0.01  |
| 8     | F8           | 6.44±0.01  |

± SD (n=3)

**Fig. 3.** A) Globule size distribution in microemulsion formulation B) Zeta potential of microemulsion formulation

**Fig. 4.** Phase separation of microemulsion
preparations because it influences spreadability and drug release as well as jellification. The viscosities of all developed microemulsions were measured at 25°C at 12 rpm using spindle s-18. M1 formulation showed higher viscosity of 126.2 cps which could be due to presence of larger amount of oil phase. M4 formulation showed least viscosity of 104 cps. The viscosity had an inverse relationship between concentration of oil and S/Cos. There is no phase separation of microemulsion after centrifugation at 3000 rpm for 1 hr. as shown in Figure 4 and was confirmed stability of microemulsion. pH determination was done by using Digital pH meter and all formulations were detected in range of 6.6 to 6.8. Thus obtained pH of developed formulations are well suited for pH of skin.

**Stability studies microemulsion**

Accelerated stability study was performed for 3 months of formulations at temperature 40±2°C & 75±5% RH. Samples were maintained for 0, 30, 60, and 90days. After storage of the specified months, the formulations were analyzed & all formulations were found stable at accelerated stability study.

**Fig. 5. Microemulsion based Emulgels viscosities**

**Fig. 6. Globule size distribution in F6 gel formulation**
Formulation Batches of Emulgels

Table 3 mentioned the summarized data of different composition of excipients for formulation development and preparation of Emulgels.

Evaluations for Microemulsion based Emulgels

Physical Appearance and determination of pH

Upon the physical observation, it was observed that all formulations detected with milky white appearance, homogeneous with no grittiness, no creaming and semi-solid in consistency. The pH values were all found in range of 6-7. All pH values are mentioned in Table 4.

Rheological behavior of gel

The rheological behavioral study was performed as per prescribed procedure in the methods section. Formulation developed with Carbopol 980 (1.5-2%) was detected with increased viscosity in prepared formulations. Figure 5

Fig. 7. In vitro diffusion study of Lornoxicam from emulgel formulations

Fig. 8. Korsmeyer-Peppas kinetic treatment for Emulgel containing Lornoxicam and diffusion kinetic models for all formulation
indicated the viscosity for different formulations. All formulations detected with shear thinning property.

**Spreadability study**

20.76 gm.cm/sec spreadability coefficient was obtained for F4 formulation. So, data is obtained because of less viscous preparation.

**Extrudability test**

As per the outcome of Extrudability test for emulgel, formulation F2, F5, And F8 were contained highest amount of Carbopol 940, Carbopol 974 and Carbopol 980 i.e. 2.0 %. From this, increase in viscosity reduces the extrudability. Prepared formulations detected with better extrudability due to low viscosity in nature.

**Globule size and Globule size distribution**

At 244 nm wavelength globule size and globule size distribution was observed. When percentage of surfactant less then less globule size will also observed. Figure 6 showed the globule size distribution of prepared emulgel formulation.

**Stability study of Emulgels**

F6 formulation was examined for accelerated stability and there is no any agglomeration or chemical instability was observed. Finally after 3 months of accelerated stability study,

| Table 5. Microemulsion based emulgels Anti-inflammatory activity |
|-----------------------|------------------|------------------|------------------|------------------|------------------|
| Sr. No | Group Taken | Paw Volume (mm) 0 (min) | Paw Volume (mm) 30 (min) | Paw Volume (mm) 60 (min) | Paw Volume (mm) 120 (min) | Paw Volume (mm) 180 (min) | Percent Inhibition |
| 1 | Control | 2.23±0.1317 | 4.26±0.2648 | 4.47±0.2973 | 4.6±0.2971 | 4.92±0.3192 | 0 |
| 2 | Standard | 2.68±0.1186 | 4.34±0.2447 | 3.8±0.2007 | 3.33±0.1549 | 3.12±0.2776 | 69.92 |
| 3 | Test | 2.25±0.0648 | 4.242±0.1814 | 3.82±0.2366 | 3.52±0.2033 | 3.38±0.2290 | 62.08 |

*(n=3)*

1) Test (Before)  
2) Control (Before)  
3) Ref. (Before)  
4) After 24 hrs.  
5) After 24 hrs.  
6) After 24 hrs.

Fig. 9. Skin irritation test conducted on albino mice for microemulsion based emulgel
developed Emulgel (F6) formulation detected with good stability and did not show any major physical/chemical changes in the emulgel.

**In-vitro permeability or diffusion test**

Figure 7 mentioned drug release pattern of drug from emulgels. Formulations F3 and F6 showed drug release in 8 hrs as 88.42 % and 90.47% respectively. Thus, formulation EG6 was detected with good in-vitro diffusion as compared to others.

**Diffusion models for Emulgel formulations**

The release kinetics of the different formulations were shown in Figure 8 by Korsmeyer-Peppas model. The release of drug followed erosion type of diffusion. As per the outcome of the study, it can be considered that the drug release was increased with increase in time exponentially. Figure number 8 indicates diffusion kinetic models for all formulation and Korsmeyer-Peppas kinetic treatment for Emulgel containing Lornoxicam.

**Skin irritation test**

Figure 9 showed the outcome of skin irritation test. Mice was used for the skin irritation test. After 24 hrs, it was observed that, there is no skin irritation such as redness or rashes on the skin. Its means prepared final formulation Emulgel can be considered as safe for the application on the skin.

**Anti-inflammatory study**

The standard group was treated with Diclofenac sodium gel, emulgel F6 formulation as test sample and carrageenan as control. 69.92 % & 62.08 % was observed percent inhibition for paw volume of emulgel for anti-inflammatory activity. < 0.0001 P value was observed by ANOVA test which showed formulation is significant. Table No 5 mentioned the outcome of anti-inflammatory activity.

**CONCLUSION**

Microemulsion system provides solubilization of hydrophobic drugs, thus imparts in enhancing the availability of the drug in the formulation. Firstly stable microemulsion was prepared and it was developed into Emulgel as a final formulation. Carbopol 980(1.5 %) gave better results as compared to other batches of emulgels. Prepared Emulgel was evaluated for their different characterization properties including, physical appearance, and pH of the formulation, rheological behaviour of the formulation, spreadability and extrudability study as well as globule size of emulgel and its distribution in the formulation. In the regards of all characterization aspects, the prepared Emulgel was shown the positive results. 90.42% drug release was detected by formulation batch F6 in 8 hr. The prepared Emulgel formulation was free from any skin irritation and detected with 62.02% inhibition of edema. Therefore, microemulsion loaded Lornoxicam emulgel gave better analgesic effect and utilized as anti-inflammatory activity.

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**Conflict of Interest**

The authors declare the no conflicts of interest

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