An ecological and multi valuable raw material for obtaining effective calcium preparations.

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Abstract: Nine tablet formulations containing calcium salts: citrate, fumarate, gluconate were prepared. Preparations containing calcium salts were made using a modified natural source of calcium, which are the chicken eggshells. Calcium amount, moisture, friability of tablets and disintegration time were determined in the formulations. The influence of the amount and type of calcium salt (citrate, fumarate), the amount of potato starch and inulin on disintegration time and half-release of calcium from tablets (t50%) was determined using the method of analysis of variance. Obtained results were compared to variant 9 containing calcium gluconate (reference). It was found that the amount and type of calcium salt (citrate, fumarate), starch and inulin amount are significant for the disintegration time of tablets. The disintegration time of tablets containing calcium fumarate is about 5 times longer compared to tablets with calcium citrate. Increasing the content of calcium citrate and inulin prolongs the disintegration time of tablets, and increasing of calcium fumarate and potato starch shortens the disintegration time. A significant effect of the composition of the tablet mass on the dynamics of calcium release from tablets has been demonstrated. Increasing the content of calcium citrate and inulin in tablets is slowing down the release rate of calcium from tablets, and increasing of fumarate and starch enhance the release rate.

Key words: calcium deficiencies, modified chicken eggshells, tableting, availability

I. Introduction

Chicken egg - a life incubator is an excellent raw material for the isolation of many biologically active substances (from the group of proteins, fats, vitamins, bioelements, etc.). Shells of chicken eggs are obtained under controlled conditions, which limits the bioaccumulation potential of xenobiotics, and at the same time allows you to model their chemical composition, enriching some of the micronutrients or increasing the content of e.g. collagen.

Eggs, a by-product of the egg industry, may be a convenient raw material for the production of calcium supplements.1,2,3,4,5 Antimicrobial, analgesic and anti-inflammatory properties of chicken eggshells were demonstrated.6,7 It also proved that the natural powder of inner shell membranes significantly decreases joint stiffness, reduces pain as well showed a positive effect on the change in bone density in postmenopausal women and in ovariectomised rat.8,9,10,11 Unfortunately calcium carbonate contained in egg shells, after oral administration causes alkalization of the body fluids and is difficult to absorb by the body. An interesting solution may be the use of calcium from shells in the form of better solubility organic salts - citrate, fumarate and gluconate for supplementation in the form of tablets.12,13,14,15 Tablets are one of the most preferred forms of the drug by patients.

The aim of this study was to determine the in vitro availability of calcium from tablets containing calcium citrate or calcium fumarate. The kinetics of calcium release from tablets obtained from modified eggshells was investigated - constant release rate (k) and half-time (t50%). The influence of the composition of the tablets – calcium salt (citrate or fumarate) and the influence of the content of potato starch and inulin on the availability were investigated. The tests used substances that are used in calcium supplementation and fortification.12,13,14,15,16,17 Calcium gluconate was a reference substance.

II. Material And Methods

2.1. Raw Materials

Crumbled, dry eggshells with membranes (Ovopol, Nowa Sól, Poland), citric acid monohydrate (Brenntag, Kędzierzyn-Koźle, Poland), anhydrous fumaric acid (POCH), anhydrous gluconic acid (Sigma), inulin (Brenntag, Kędzierzyn-Koźle, Poland), potato starch (PEPES), magnesium stearate (Chem&Pol, www.iosrjournals.org
Warsaw, Poland). All substances were analytically pure and complied with quality standards.

2.2. Preparation of calcium salts - citrate, gluconate and fumarate
A suitable calcium salt was prepared by mixing the milled and sieved 0.75mm sieve crusts in a 280 mg ratio with equimolar acid (citric or fumaric acid or gluconate). The resulting mixture was roasted at 120°C/2 h, then crushed and sieved through a 1.25 mm sieve. The conducted synthesis and the applied roasting temperature effectively inhibit the growth of bacteria and provide adequate sterility to be given to a human.12,18.

2.3. Preparation of tablets
The appropriate calcium salt was suitably sieved through a 1.25 mm mesh screen and then combined intimately and thoroughly with the other tablet mass components (potato starch and inulin) as shown in Table 1. The obtained powders mixture was sieved to unify of grains through a sieve with a mesh size of 1.25 mm. Next, magnesium stearate as a lubricant was added and that kind of mass was tabletted. The tablets were pressed under the same conditions - using an Erweka impact tablet machine (type AR 400), equipped with 12 mm diameter concave punches, with a constant force of 40 kN.12. On the basis of the preliminary tests, the experiment planning matrix was determined, which is presented in Table No 1.

Table No 1: Plan of the experiment (composition per 1 tablet).

<table>
<thead>
<tr>
<th>Variant</th>
<th>The type of Calcium salt</th>
<th>Calcium salt [mg]</th>
<th>Potato starch 1/[%]</th>
<th>Inulin 1/ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>[+}Citrate</td>
<td>+}447</td>
<td>+}12</td>
<td>-}4</td>
</tr>
<tr>
<td>F-2</td>
<td>[-}Fumarate</td>
<td>-}376</td>
<td>+}12</td>
<td>+}12</td>
</tr>
<tr>
<td>F-3</td>
<td>[+}Citrate</td>
<td>+}370</td>
<td>+}12</td>
<td>-}4</td>
</tr>
<tr>
<td>F-4</td>
<td>[-}Fumarate</td>
<td>+}578</td>
<td>+}12</td>
<td>+}12</td>
</tr>
<tr>
<td>F-5</td>
<td>[+}Citrate</td>
<td>+}447</td>
<td>-}4</td>
<td>-}4</td>
</tr>
<tr>
<td>F-6</td>
<td>[-}Fumarate</td>
<td>-}376</td>
<td>-}4</td>
<td>+}12</td>
</tr>
<tr>
<td>F-7</td>
<td>[+}Citrate</td>
<td>-}370</td>
<td>-}4</td>
<td>-}4</td>
</tr>
<tr>
<td>F-8</td>
<td>[-}Fumarate</td>
<td>+}578</td>
<td>-}4</td>
<td>+}12</td>
</tr>
<tr>
<td>F-9</td>
<td>Gluconate</td>
<td>431</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

[+} – upper level
[-} - lower level
1/ - percent of starch/inulin calculated in relation to the calcium salt content.

Table No 2 presents the composition of the tablet mass (per one tablet). 9 formulations were made and each was repeated 5 times.

Table No 2: Composition of the tablet mass (per 1 tablet).

<table>
<thead>
<tr>
<th>Variant</th>
<th>The type of calcium salt</th>
<th>Calcium salt [mg]</th>
<th>Potato starch [mg]</th>
<th>Inulin [mg]</th>
<th>Magnesium stearate [mg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>Citrate</td>
<td>447</td>
<td>53.4</td>
<td>17.9</td>
<td>13.0</td>
</tr>
<tr>
<td>F-2</td>
<td>Fumarate</td>
<td>376</td>
<td>45.1</td>
<td>45.1</td>
<td>11.7</td>
</tr>
<tr>
<td>F-3</td>
<td>Citrate</td>
<td>370</td>
<td>44.4</td>
<td>14.8</td>
<td>10.7</td>
</tr>
<tr>
<td>F-4</td>
<td>Fumarate</td>
<td>578</td>
<td>69.4</td>
<td>69.4</td>
<td>17.9</td>
</tr>
<tr>
<td>F-5</td>
<td>Citrate</td>
<td>447</td>
<td>17.9</td>
<td>17.9</td>
<td>12.1</td>
</tr>
<tr>
<td>F-6</td>
<td>Fumarate</td>
<td>376</td>
<td>15.0</td>
<td>45.1</td>
<td>10.9</td>
</tr>
<tr>
<td>F-7</td>
<td>Citrate</td>
<td>370</td>
<td>14.8</td>
<td>14.8</td>
<td>10.0</td>
</tr>
<tr>
<td>F-8</td>
<td>Fumarate</td>
<td>578</td>
<td>23.1</td>
<td>69.4</td>
<td>16.8</td>
</tr>
<tr>
<td>F-9</td>
<td>Gluconate</td>
<td>431</td>
<td>34.5</td>
<td>34.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

2.4. Physicochemical properties of tablets
Obtained tablets were investigated for physicochemical properties according to the Polish and European Pharmacopoeia.12,13,19,20. Mean mass (mg), calcium content (mg), moisture content [%], friability [%] and disintegration time [min] were determined.

2.4.1. Calcium content determination
To determine the amount of calcium(II) ions a validated spectrophotometric method was used (Calcium O-CPC Kit; Pointe Scientific, Canton, USA). It is based on the reaction of calcium ions with o-cresolphthalein complexone (CPC) in the alkaline environment. The intensity of colour was measured with a UV-VIS „Marcel
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Media" spectrophotometer (France) in 1.0 cm glass cuvettes at the wavelength of λ = 570 nm. The photometric accuracy of the spectrophotometer was ± 0.005 A. The empirical regression equation y = 0.0585 x - 0.0001 was used to establish the relationship between calcium ion content and absorbance. The significance of the equation was $R^2 = 0.9974$; $p < 0.01$; linearity up to 20 mg / dL.

2.4.2. Moisture content determination
Humidity was determined in a WS-30 moisture analyzer (Radwag, Poland). ~ 5 g of shredded tablet mass was spread evenly with a thin layer over the whole surface of the pan and dried until a constant weight was obtained.

2.4.3. Friability
The weight of 20 tablets was accurately determined before and after rotation in the drum of a tablet friability test apparatus (EF-2W, Electrolab, Mumbai, India) for 4 minutes at 25 rpm. The difference in weight indicates the rate of friability (%). A maximum loss of 1 percent of the mass of the tablets tested is considered to be acceptable for uncoated tablets.

2.4.4. Disintegration time
Disintegration time (n = 6) was determined in 500 mL of 0.1 M HCl at 37±2°C with the use of DisiTest20 (Sotax, Switzerland). Tablets were placed separately in tubes which were limited from the bottom with a sieve and burdened from the top with cylindrical rings. The tube with the tablet was moved up and down through the distance of 55 ± 2 mm at a frequency of 30 cycles per minute. Uncoated tablets comply with the test if all six have disintegrated within a period of not more than 15 minutes.

The effect of the content and type of calcium salt (citrate, fumarate) and the content of potato starch and inulin on the disintegration time using the analysis of variance method was determined. The existing interdependencies between the composition of the tablets and the time of their disintegration were examined.

2.5. Dissolution test in vitro
The test was performed in Erweka DT-600 paddle apparatus (Germany). The rate of calcium release from 6 tablets was measured for 5 h (37 ± 0.5°C, 100 rpm) using 500 mL of artificial gastric acid (0.1 M/L hydrochloric acid, pH = 1.2) The samples in the amount of 3 ml were collected in 0.5 h periods 0.5 h, and filtered through the filter (0.45 μm pores). The amount of release calcium in the collected samples was determined.

Based on the obtained results, it was found out that calcium release showed the first-order kinetics. The parameters of this process such as calcium release rate constant (k) and half-time of calcium release ($t_{50\%}$) were determined. Obtained results were compared to variant 9 (reference) containing calcium gluconate.

2.6. Results analysis
The percentage of released calcium in a time unit was determined and the release profiles were plotted. Differences in the calcium release were evaluated by calculating the similarity coefficients ($f_2$) and difference ($f_1$). Statistica "Pharmaceutical Kit: Release Profiles” was used to analyze the results - version 2.7 (StatSoft Poland Sp. z o.o.). The results were calculated as mean values (± SD) and were analyzed statistically using the Microsoft Excell package and Statistica (StatSoft, Inc) option: Industrial analysis, experimental design (DOE). The Statistica Pharmaceutical Kit: Statistica "Release Profiles” The Weibull distribution methodology was used to analyze the results.. The statistically significant significance at the level of $p <0.05$ was calculated using the Student's t test.

The influence of content and calcium salt (citrate, fumarate) and the content of potato starch and inulin for the half-time of calcium release ($t_{50\%}$) were determined using the analysis of variance. The existing interdependencies between the composition of the tablets and the half-time of calcium ($t_{50\%}$) were determined.

### III. Results and discussion

The content of calcium in tablets ranges from 98.3 mg (F-7) to 178.7 mg (F-8). The weight of the tablets is in a range 409.6 mg (F-7) - 734.7 mg (F-4). The water content in tablets is from 1.00% to 2.76%, and friability from 0.26% (F-1) to 10.49% (F-7). Only F-1 and F-5 tablets (with calcium citrate) comply with pharmacopeial requirements. F-8 tablets (calcium fumarate, 4% starch, 12% inulin) have similar mechanical resistance to tablets F-9 (calcium gluconate).

Table No 3 presents the physico-chemical properties of obtained tablets.
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Table No 3: Characteristics of tablets with calcium citrate or calcium fumarate or calcium gluconate.

<table>
<thead>
<tr>
<th>Tablets</th>
<th>Calcium content [mg]</th>
<th>Tablet mass [mg]</th>
<th>Water content [%]</th>
<th>Friability [%]</th>
<th>Disintegration time [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>127.5± 0.30</td>
<td>551.3</td>
<td>2.76±0.21</td>
<td>0.26±0.08*</td>
<td>0.00±0.51*</td>
</tr>
<tr>
<td>F-2</td>
<td>124.3± 3.14</td>
<td>447.9</td>
<td>2.31±0.03</td>
<td>5.32±0.82*</td>
<td>11.02±0.76*</td>
</tr>
<tr>
<td>F-3</td>
<td>105.6±2.63</td>
<td>439.9</td>
<td>2.01±0.02</td>
<td>3.86±0.81*</td>
<td>1.47±0.29*</td>
</tr>
<tr>
<td>F-4</td>
<td>191.0±3.52</td>
<td>734.7</td>
<td>1.00±0.18</td>
<td>6.22±1.02*</td>
<td>12.83±0.76*</td>
</tr>
<tr>
<td>F-5</td>
<td>118.8±1.16</td>
<td>494.9</td>
<td>1.54±0.40</td>
<td>0.96±0.12*</td>
<td>6.75±0.75</td>
</tr>
<tr>
<td>F-6</td>
<td>116.2±1.87</td>
<td>447.0</td>
<td>1.64±0.11</td>
<td>4.65±0.92*</td>
<td>27.33±2.52*</td>
</tr>
<tr>
<td>F-7</td>
<td>98.3±2.03</td>
<td>409.6</td>
<td>1.02±0.03</td>
<td>10.49±0.98*</td>
<td>0.42±0.14*</td>
</tr>
<tr>
<td>F-8</td>
<td>178.7±0.95</td>
<td>687.3</td>
<td>1.16±0.08</td>
<td>3.43±0.93</td>
<td>12.08±1.01*</td>
</tr>
<tr>
<td>F-9</td>
<td>40.0±0.65</td>
<td>512.5</td>
<td>1.57±0.03</td>
<td>3.02±0.72</td>
<td>7.17±1.04</td>
</tr>
</tbody>
</table>

* - statistically significant in relations to F-9 (reference).

The tablet disintegration time is in a range 0.42 min (F-7) - 27.33 min (F-6). All formulations except F-6 were disintegrating in <15 minutes. The disintegration time of all formulations except for F-5 is statistically significantly different from F-9 (calcium gluconate). The obtained results indicate that the disintegration time of tablets containing calcium fumarate (~ 16 minutes) is ~ 5 times longer as compared to tablets with calcium citrate (~ 3 minutes). Increasing the content of calcium citrate in tablets prolongs their disintegration time ($r^2 = 0.725$). However, with the increase of calcium fumarate content in tablets, their disintegration time decreases ($r^2 = -0.503$). The disintegration time of tablets containing 12% starch is ~ 7 minutes, and containing - 4% increases to ~ 12 minutes. Tablets containing 12% inulin have a ~ 5 times longer disintegration time compared to tablets containing 4% of this substance. The higher the content of inulin in the tablets, the longer their disintegration time ($r^2 = 0.632$). In the studied range, there was no correlation between the content of starch in the mass of tablets and their disintegration time. The content and the type of calcium salt (citrate, fumarate) in tablets and the content of inulin (p <0.05) have a significant influence on the time of tablets disintegration.

The dynamics of calcium release from tablets containing calcium citrate and gluconate (variant 9 - reference) is shown in Figure 1.

![The calcium release profile](image-url)

Figure 1. The effect tablet mass composition on calcium release from tablets, containing calcium citrate and gluconate.

* - statistically significant in relations to F-9 (reference).
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The Weibull method (p <0.05) showed significant statistical differences in calcium release profiles from tablets containing calcium citrate from eggshells (F-1, F-5 and F-7) except for the F-3 formulation compared to its release from tablets with calcium gluconate (F-9). This indicates a significant influence of the composition of the tested calcium preparations on the dynamics of calcium release. The amount of calcium released from tablets containing modified shells in the form of citrate and gluconate is different, especially in the initial phase. The entire dose of calcium from calcium citrate (F-5 and F-7) formulations is released in 1 hour. The obtained results suggest a quick supplementation of calcium deficiency in the body when it is given in the form of citrate. A mild increase in calcium release was observed on the other hand from other formulations (F-1, F-3 and F-9), which may suggest a gradual supplementation of its deficit in the body without its overdose. It seems reasonable that during oral supplementation with calcium salts it is recommended to administer them in lower doses up to 500 mg. In the in vivo model studies, it was shown that the most calcium is permeated and absorbed by the model membranes (small intestine) after administration of lower doses. It was also reported that calcium administration at a dose of 200 mg 4 times a day significantly reduced the concentration of PTH which prevented bone resorption. The release of calcium in vitro indicates its ability to absorb in vivo.

Figure 2 presents the dynamics of calcium release from tablets containing fumarate and calcium gluconate (variant 9 - reference).

Significant statistical differences were found in calcium release profiles from tablets containing calcium fumarate (F-2, F-6, F-8) compared to its release from tablets containing calcium gluconate (F-9). There was a smooth increase in released calcium from all formulations except for F-4, where the whole dose of calcium was released within 1 h. Obtained results suggest that calcium released from tablets containing calcium fumarate and calcium gluconate may gradually supplement its deficit in the body. Calcium fumarate contains 26% calcium in the molecule, citrate 24%, and gluconate is 9.3%. In the group of dialysis patients with chronic renal failure who have calcium-phosphate disturbances it was found that calcium fumarate is more effective, better tolerated and free from adverse effects compared to calcium carbonate. The obtained results are

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confirmed by studies in which the penetration of calcium through the small intestine was simulated depending on the type of calcium salt, its concentration and the pH value of the acceptor environment. Adequate conditions of absorption of calcium salts and the composition of tablets may ensure greater availability and thus greater effectiveness during supplementation.\textsuperscript{12,13,14,16} There are many calcium supplements available on the market that contain calcium of natural or synthetic origin, which releases calcium at various rates.\textsuperscript{13}

Table No 4 presents the parameters characterizing the pharmaceutical availability of calcium in vitro conditions depending on the composition of tablets.

### Table No 4: Parameters of calcium pharmaceutical availability from tablets depending on the composition

<table>
<thead>
<tr>
<th>Tablets</th>
<th>The amount of calcium release [%]</th>
<th>Constant release rate k [h(^{-1})]</th>
<th>Half-time t(_{50%}) [h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.96 ± 0.23</td>
<td>1.36 ± 0.06*</td>
<td>0.51 ± 0.02*</td>
</tr>
<tr>
<td>2</td>
<td>96.53 ± 3.60</td>
<td>0.57 ± 0.05*</td>
<td>1.23 ± 0.11*</td>
</tr>
<tr>
<td>3</td>
<td>99.76 ± 2.52</td>
<td>0.70 ± 0.07</td>
<td>1.01 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>within 1 h</td>
<td>1.80 ± 0.07*</td>
<td>0.39 ± 0.01*</td>
</tr>
<tr>
<td>5</td>
<td>within 1 h</td>
<td>3.38 ± 0.33*</td>
<td>0.21 ± 0.02*</td>
</tr>
<tr>
<td>6</td>
<td>79.09 ± 1.77</td>
<td>0.25 ± 0.04*</td>
<td>2.82 ± 0.39*</td>
</tr>
<tr>
<td>7</td>
<td>Do 1 h</td>
<td>6.21 ± 1.58*</td>
<td>0.12 ± 0.03*</td>
</tr>
<tr>
<td>8</td>
<td>86.99 ± 0.69</td>
<td>0.22 ± 0.01*</td>
<td>3.12 ± 0.08*</td>
</tr>
<tr>
<td>9</td>
<td>95.52 ± 0.94</td>
<td>0.61 ± 0.03</td>
<td>1.12 ± 0.03</td>
</tr>
</tbody>
</table>

* - statistically significant in relations to F-9 (reference).

Calcium is released from all formulations according to 1st order kinetics. It is a kinetic model characteristic of conventional forms.\textsuperscript{12,13} The release time of calcium from tablets is from 0.12 h (F-7) to 3.12 h (F-8). Compared to the release rate of calcium from tablets containing gluconate (F-9), significant statistical differences in the rate of its release from all tablets except the F-3 formulation were found. Calcium from tablets with citrate (F-7) is the most rapidly releasing, and the slowest one from tablets containing calcium fumarate (F-8). The half-life of calcium (~ 0.46 h) from tablets containing calcium citrate is four times shorter than tablets with calcium fumarate (~ 1.89 hours). Increased starch content (12%) in tablets shortens \( t_{40\%} \) - ~ 2 times, compared to tablets containing 4% starch. Inulin added to tablets at a concentration of 12% increases the calcium release half time ~ 4 times compared to tablets containing 4% inulin. The type of salt (citrate, fumarate), starch and inulin content (p <0.05) has a significant influence on the release time of calcium from tablets. The more inulin in the composition of the tablets is, the slower the release of calcium (\( r^2 = 0.632 \)). In the studied range, no correlation was found between the other components of the tablet mass and the calcium half-time.

It has been reported that the bioavailability of calcium from eggshells is greater than that of calcium carbonate, and the release of calcium from tablets containing modified chicken egg shells in the form of citrate is faster than from synthetic calcium carbonate.\textsuperscript{12}

Dietary supplements obtained on the basis of powdered egg shells supplement not only calcium deficiencies of the body, but also provide the body with microelements, such as copper, selenium, fluorine and strontium, which have a positive effect on bone metabolism, stimulating osteoblasts and inhibiting the resorption activity of osteoclasts.\textsuperscript{12,3,4,5,6,7,8,9,10} It suggests for carbonate to be recommended to people with normal stomach acidity and hyperacidity, and fumarate and gluconate - for people with hypocrisy. Calcium citrate may be available to the body regardless of the pH value of the site of action.\textsuperscript{14,16}

### IV. Conclusion

The use of chicken eggshells (waste of the egg industry) may be a more effective alternative to calcium sources currently used in human and animal supplementation.

Tablets containing calcium citrate obtained from modified shells can quickly supplement calcium deficiency in the body, and fumarate-containing tablets will gradually supplement its deficit.

A significant effect of the composition of the tablet mass on the dynamics of calcium release from tablets has been demonstrated.

The content and type of calcium salt (citrate, fumarate) and the content of inulin and starch have a significant effect on the disintegration time. The disintegration time of tablets containing calcium fumarate is ~ 5 times longer compared to calcium citrate tablets. Increasing the content of calcium citrate and inulin in tablets prolongs their disintegration time, and fumarate and starch shortens disintegration time.

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The type of salt (citrate, fumarate), potato starch and inulin have a significant influence on the release of calcium from tablets. Increasing the content of calcium citrate and inulin in tablets shows slower release of calcium and fumarate and starch accelerates.

Calcium citrate obtained from sterile chicken eggshells can be an invaluable raw material for obtaining tablets. It allows for quick and effective supplementation of calcium deficiencies in the body.

Acknowledgements

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Human and animal rights

No Animals/Humans were used for studies that are the basis of this research.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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