Dysmenorrhea Clinical Pharmacology: Dose Finding of Physiological Modulators in Foods or Capsules

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ABSTRACT

**Background:** Dysmenorrhea is a disease, which occurs in about 45% of women in childbearing age.

**Objective:** Dose finding study to evaluate the activity of specific physiological modulators (PMs) aimed at protecting GABA_A receptors and CaSRs (calcium sensing receptors) against oxidative stress (OS) and at reducing the discomfort caused by dysmenorrhea.

**Methods:** The discomfort was measured using a score of from 0 to 5 and plasma antioxidant capacity was determined using a plasma antioxidant test (PAT). The PMs were combined and administered at low doses in the form of specific foods (SFs) and capsules. Twenty women with a score of ≥ 3 took part in the study during 5 sessions of 4 hours.

In session 1, the women were treated with placebo, whereas in sessions from 2 to 5 they alternated between capsules and SFs containing the same amount of PMs. In sessions 4 and 5, the PM doses were double those taken in sessions 2 and 3. At the end of each sessions, women were asked about the need of pain relief as an indirect measurement of treatment efficacy.

**Results:** The SFs and capsules reduced the discomfort in 60% and 75% of cases respectively. However, the effect with capsules was more rapid and lasted longer than with SFs. The need for pain relief was lower after treatment with capsules than it was with SFs. The increase in PAT was similar for both treatments.

**Conclusions:** PMs taken as SFs or capsules showed similar activity, but capsules had a significantly more rapid and longer lasting effect.

**Keywords**
Physiological modulators, Dysmenorrhea, Daily discomfort, Specific foods, Plasma antioxidant capacity, Oxidative stress.

**Introduction**
Physiological Modulators (PMs) have been already shown to reduce the symptoms of dysmenorrhea when given in capsules [1]. In particular, their activity seems due to the reduction of the oxidative stress (OS) in the gastrointestinal tract (GI) and in the central nervous system (CNS). At GI level, there are several calcium sensing receptors (CaSRs), while in the CNS are more represented the GABA_A receptors. In both cases, the presence of cysteine loops, respectively in the ionic channels of GABA_A structure and in the ECD (Extra Cellular Dominion) of CaSRs, makes these receptors very sensible to the OS, which opens the double bonds of cysteines and makes inefficient some of the proteins responsible for the receptors activity. The damage of these receptors -who control the release of several mediators-
can generate most of the somatic and behavioral symptoms of dysmenorrhea, which were controlled with the use of PMs. By definition, PMs are natural compounds that can be taken with either foods or food supplements [2].

What is most important is the dosage, because an excessive intake can generate an increase of OS instead of a decrease [3-4]. However, in previous studies it was not defined what could be the effect when PMs are given in capsules or simply as foods [1]. The present study is aimed to compare the activity of PMs taken with food or as supplements for the reduction of the dysmenorrhea discomfort.

**Methods**

The first step was to determine the type and the quantity of the PMs that may be used. The products were chosen based on the pharmacological and bioavailability characteristics of each component known to have a specific antioxidant activity.

- **Citrus bioflavonoids:** to reduce the OS in the GI tract and protect the ionotropic GABA$_A$ receptors and CaSRs [5-7].

- **Lycopene:** to protect lymphocytes from oxidative damage and limit the transfer of oxidation into the circulation and tissues [8].

- **Astaxanthin:** to protect neurons from oxidative stress [9]. Calcium lactate and calcium carbonate: to make calcium available to the CaSRs in the GI tract [10].

- **Vitamin D$_3$:** to improve calcium absorption. The second step was the dosage of each PMs. The dosages of PMs (citrus bioflavonoids, lycopene and astaxanthin) was determined to provide an antioxidant capacity measured *in vitro* using PAT (Plasma Antioxidant activity Test) [11,12] of at least 500 U. Cor. for each component. The total combined antioxidant capacity was 1500 U. Cor. In terms of ascorbic acid, a value of 1500 U.Cor. *in vitro* can be achieved with about 270-280 mg. The quantities of the products to obtain 500 U. Cor. *in vitro* are reported in Table 1.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Measure</th>
<th>U.Cor. as mEq vitamin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus bioflavonoids</td>
<td>13.3</td>
<td>mg</td>
<td>500</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.4</td>
<td>mg</td>
<td>500</td>
</tr>
<tr>
<td>Astaxanthin</td>
<td>0.1</td>
<td>mg</td>
<td>500</td>
</tr>
<tr>
<td>Vitamin D$_3$</td>
<td>1.0</td>
<td>mcg</td>
<td>0</td>
</tr>
<tr>
<td>Calcium</td>
<td>100.5</td>
<td>mg</td>
<td>0</td>
</tr>
</tbody>
</table>

**Calcium:** was necessary to activate CaSRs, and the dosage was chosen based on the daily calcium excretion with urine (between 100 to 300 mg in 24 h). The calcium was used as a combination of lactate and carbonate salts (respectively in the quantities of 143 and 209 mg). For Vitamin D$_3$, the dosage of 1 mcg was chosen to administer 1/10 of the RDA (Recommended Daily Allowance).

**The foods composition**

The quantities of PMs were identical in capsules and SFs. Values are reported in Table 2 and Figure 1.

**Figure 1:** Example of SFs.

Legenda: Craker (10g) is reported in the lower part of the figure with the salmon (8g) with small lice of tomato (3g) on top, together with a teaspoon of olive oil (2 g), and parmesan cheese (9 g). The glass red orange juice (150 mL), made with fresh oranges is in the upper part of the figure.

The content of calcium was about 128 mg.

The Placebo food (PFs) was prepared also, since it was necessary for a correct comparison. Despite a higher total weight, the number of calories was identical to the SFs. The components were selected for the lack (or traces only) of the PMs contained in the capsules. The composition of the PFs is reported in Table 3 and Figure 2.

**Figure 2:** Example of the PFs.
Legenda: one cracker (10 g) is reported in the lower part with the endive on the top (10 g); boiled potato in slices (140 g) are in the cup; a teaspoon of olive oil (2 g). The glass of mineral water (150 mL with calcium content < 5 mg/L) is shown in the upper part of the figure. The content of calcium in PFs was about 17 mg.

SFs and PFs were prepared by the same restaurant; the women added oil now of the intake.

Evaluation of the antioxidant capacity (PAT or plasma antioxidants test)
This variable measures the antioxidant capacity of plasma in terms of mEq of ascorbic acid (U. Cor.) [12].

Briefly: Each food or component was homogenized (mini-homogenizer Cheimika-Italy) with 50 mL of distilled water for 5 min, and 10 µL of the suspension were used for the evaluation. The orange juice was used without any dilution. The total values of PAT adding the values of all components was 1500 U. Cor. and none was lower than 300 U. Cor. a part of olive oil. Appropriate kits were used for the evaluation [11]. PAT was determined on the blood taken with a tip finger and collected in a micro cuvette containing heparin. Plasma was isolated by centrifugation (90 second at 800 G), and the evaluation was done using 10 µL. The measures were taken at the enrolment (at 10 am in the Session 0 see later), and two hours after the intake of foods or capsule in all the other sessions (from 1 to 5).

Evaluation of the discomfort
A score to measure the discomfort (Table 4) was used as admission criteria (score ≥ 3), and in the days of the treatments before and, 1, 2, 4 hours after the intake of the PFs, SFs or capsule/s containing PMs. The improvement of the discomfort was considered significant only in case of 2 points of difference in comparison to the baseline value.

Table 2: Specific foods (SFs) containing the same amount of 1 capsule of PMs.

<table>
<thead>
<tr>
<th>Food</th>
<th>Quantity</th>
<th>kcalories</th>
<th>Type of PMs</th>
<th>Quantity of specific PMs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Orange Juice</td>
<td>150 mL</td>
<td>51</td>
<td>Citrus bioflavonoids</td>
<td>15 mg</td>
<td>13</td>
</tr>
<tr>
<td>Tomato</td>
<td>4 g</td>
<td>1</td>
<td>Lycopene</td>
<td>0.4 mg</td>
<td>14</td>
</tr>
<tr>
<td>Farmed salmon</td>
<td>8 g</td>
<td>15</td>
<td>Astaxanthin calcium</td>
<td>0.1 mg</td>
<td>15</td>
</tr>
<tr>
<td>Farmed salmon</td>
<td>8 g</td>
<td>34</td>
<td>Calcium</td>
<td>100.5</td>
<td>17</td>
</tr>
<tr>
<td>Parmesan cheese</td>
<td>9 g</td>
<td>43</td>
<td>Calcium</td>
<td>2 mg</td>
<td>17</td>
</tr>
<tr>
<td>Cracker (white bread)</td>
<td>10 g</td>
<td>18</td>
<td>Other than required</td>
<td>traces</td>
<td>17</td>
</tr>
<tr>
<td>Olive oil</td>
<td>2 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total calcium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>128 mg</td>
</tr>
<tr>
<td><strong>PAT</strong></td>
<td></td>
<td>mEq</td>
<td>ascorbic acid</td>
<td>1500</td>
<td>162</td>
</tr>
</tbody>
</table>

Table 3: Placebo foods (PFs) composition [17].

<table>
<thead>
<tr>
<th>Food</th>
<th>Quantity</th>
<th>kcalories</th>
<th>Type of specific PMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral water a</td>
<td>150 mL</td>
<td>0</td>
<td>calcium &lt; 1 mg</td>
</tr>
<tr>
<td>Boiled potato</td>
<td>140 g</td>
<td>99</td>
<td>calcium 9 mg; traces of the other PMs</td>
</tr>
<tr>
<td>Cracker (white bread)</td>
<td>10 g</td>
<td>43</td>
<td>Calcium 2 mg; traces of the other PMs</td>
</tr>
<tr>
<td>Olive oil</td>
<td>2 g</td>
<td>18</td>
<td>Traces of the other PMs</td>
</tr>
<tr>
<td><strong>Total calcium</strong></td>
<td></td>
<td></td>
<td>&lt; 17 mg</td>
</tr>
<tr>
<td><strong>PAT</strong></td>
<td>mEq</td>
<td></td>
<td>&lt; 300, under sensitivity limits in most of the components</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>272 g</td>
<td>162</td>
<td></td>
</tr>
</tbody>
</table>

a = Calcium content < 5 mg/L

Legenda: one cracker (10 g) is reported in the lower part with the endive on the top (10 g); boiled potato in slices (140 g) are in the cup; a teaspoon of olive oil (2 g). The glass of mineral water (150 mL with calcium content < 5 mg/L) is shown in the upper part of the figure. The content of calcium in PFs was about 17 mg.

SFs and PFs were prepared by the same restaurant; the women added oil now of the intake.

Evaluation of the antioxidant capacity (PAT or plasma antioxidants test)
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Briefly: Each food or component was homogenized (mini-homogenizer Cheimika-Italy) with 50 mL of distilled water for 5 min, and 10 µL of the suspension were used for the evaluation. The orange juice was used without any dilution. The total values of PAT adding the values of all components was 1500 U. Cor. and none was lower than 300 U. Cor. a part of olive oil. Appropriate kits were used for the evaluation [11]. PAT was determined on the blood taken with a tip finger and collected in a micro cuvette containing heparin. Plasma was isolated by centrifugation (90 second at 800 G), and the evaluation was done using 10 µL. The measures were taken at the enrolment (at 10 am in the Session 0 see later), and two hours after the intake of foods or capsule in all the other sessions (from 1 to 5).

Evaluation of the discomfort
A score to measure the discomfort (Table 4) was used as admission criteria (score ≥ 3), and in the days of the treatments before and, 1, 2, 4 hours after the intake of the PFs, SFs or capsule/s containing PMs. The improvement of the discomfort was considered significant only in case of 2 points of difference in comparison to the baseline value.

Table 4: Symptom scores and relative severity.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description of severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No discomfort</td>
</tr>
<tr>
<td>1</td>
<td>Very mild, compatible with normality</td>
</tr>
<tr>
<td>2</td>
<td>Mild, noticeable but not interfering with normal activity</td>
</tr>
<tr>
<td>3</td>
<td>Moderate with some limitation of normal activity</td>
</tr>
<tr>
<td>4</td>
<td>Severe with strong limitation of normal activity</td>
</tr>
<tr>
<td>5</td>
<td>Intolerable, not allowing any activity</td>
</tr>
</tbody>
</table>

Experimental procedure
All the Session of the study were conducted in 5 consecutive months.

The admission (Session 0) consisted of the evaluation of the discomfort score and PAT two days after the monthly suspension of OC during menses and following an overnight fasting. Only women with anamnetic and present scores ≥ 3 were accepted.

In the Session 0, the food intake was controlled according to FIA (food intake assessment) [18] to determine the average daily intake of calcium for each of the following Sessions (from 1 to 5, see Table 4) the evaluation started two days after the OC withdrawal, during menses and the discomfort was measured at baseline and 1, 2 and 4 hours after treatments.

The Session 1 consisted of the intake of food not containing any
of the specific PMs or PFs (Placebo foods) together with and empty capsule identical to the capsule containing PMs. This was necessary to measure the placebo effect in terms of both foods and capsules. In the Session 2, the women were treated with one capsule containing PMs, and in the Session 3 with one SFs. The administration of two SFs and two capsules followed respectively in the Session 4 and 5. No pain rescue treatment was allowed during the 4 hours of the evaluation. At the end of each Session, the women were free to take pain rescues and report the need to the investigators. This last variable was considered as an indirect index of the treatment efficacy. PAT was measured in all the Sessions at 10 am ± 10 minutes after the overnight fasting.

In the case of Session 1 to 5 the time was corresponding to two hours after the treatment. The experimental procedure is summarized in Table 5.

Table 5: Experimental procedure: B = baseline.

<table>
<thead>
<tr>
<th>Session</th>
<th>Treatment</th>
<th>Evaluations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session 0</td>
<td>No-Baseline</td>
<td>Score ≥ 3; FIA ; PAT at 10 am</td>
</tr>
<tr>
<td>Session 1</td>
<td>PFs + 1 empty capsule</td>
<td>Score at B, 1.2 4 hours; PAT at 10 am</td>
</tr>
<tr>
<td>Session 2</td>
<td>1 capsule of PMs</td>
<td>score at B, 1.2 4 hours; PAT at 10 am</td>
</tr>
<tr>
<td>Session 3</td>
<td>1 SFs</td>
<td>score at B, 1.2 4 hours; PAT at 10 am</td>
</tr>
<tr>
<td>Session 4</td>
<td>2 capsules of PMs</td>
<td>score at B, 1.2 4 hours; PAT at 10 am</td>
</tr>
<tr>
<td>Session 5</td>
<td>2 SFs</td>
<td>score at B, 1.2 4 hours; PAT at 10 am</td>
</tr>
</tbody>
</table>

The evaluations were done in the morning between 8 am and 12 am in the period between April and August 2009 in Milan-Italy. The women were invited in groups of no more than four. The location was consisting of 3 rooms, with separate space for reading, watching the television or listening the music. The rooms temperature was kept at 23 °C. A small garden just out of the rooms was available for walking. The score were calculated with the aid of the investigator only during the session 0. For all the other sessions, it was self-evaluations. Foods were prepared by the same restaurant according to the prescriptions.

Subjects
Twenty-six women were enrolled.

Admission criteria
The age range was between 24-29 years, and the age of menarche was not considered. The admission criteria were the presence of an anamnestic and present score ≥ 3. All the cases were under the same OC at least from three months. This criterion was necessary because the symptoms usually appear for few days between the suspension of the pill and the starting of the menses. The same women were following the 5 sessions of the evaluation.

Exclusion criteria
Women suffering from any chronic disease were excluded. The intake of more than two portions/week of milk, cheese, dry fruit and foods containing more than 100 mg/100g of calcium was also within the exclusion criteria. This was necessary because calcium was an important PM that was part of the foods and capsules to be tested. Most of the cases were working in the medical field (nurses, doctors, pharmacists).

Statistical Evaluation
The mean scores were compared using the Tukey HSD test, and for the pain rescue use the Chi square with Yates correction was calculated. For the PAT measure, the Randomized Block Design Anova was used. The number of cases was determined in heuristic terms, because any power application was possible in terms of discomfort. However, in the case of PAT evaluation, a number of 15 cases is sufficient for a (1-β) 0.8 with and α 0.05. The JMP 14 SAS institute was used.

Ethical Committee
For the food supplements, any Ethical Committee is requested. However, the experience was approved by the IAPS (International Agency for Pharma Standards) Ethical Committee [19].

Results
The study was completed in 20 women only because 6 were abandoning the research after the Session 0 for logistic reasons, due to the difficulty to dedicate the entire morning to complete the Sessions. The daily calcium intake measured with FIA was 900 mg and, in few women only it was exceeding 1100 mg. The results are reported in Table 6.

For the Tukey test the R square was 0.8225 and for Anova the blocks value was p =0.0029, indicating in both cases a correct evaluation.

The request of pain rescues after one cps of PMs was lower than after PFs and one SFs. After the treatment with two capsules and two SFs, the values were lower than following PFs although not very different from each other. However, the request after the treatment with two capsules or two SFs were significantly lower than with one capsule or one SFs (Chi square Yates p< 0.05).

The Antioxidant activity
PAT values were significantly lower for all the treatments. The two treatments (SFs and capsules) were more effective than one treatment. No difference was shown between capsules and SFs.

Discussion
The limit of the study is the evaluation of the discomfort, which cannot discriminate between the activities on somatic or behavioral symptoms of dysmenorrhea. However, it can be considered a valid index of symptoms modification. The other limitation is the calcium intake during the day of the Sessions. The women who participate to the study were not use to drink milk or to eat dry fruits and other foods known to contain calcium for > 100 mg/100 g. The water intake during the Sessions was adding no more than 0.75-1 mg of calcium. One may not exclude those other foods taken in the days before the Sessions could in contain some more calcium.
According to the FIA, the average daily amount of calcium intake was less than 900 mg in almost all the women, and the treatment with PMs in the amount of two capsules or 2 SFs was increasing the calcium intake of about of 200-220 mg/day. Calcium intake is important, and it has already shown that 1g of calcium with or without the Vitamin D, addition can reduce the dysmenorrhea symptoms [20], and confirms that the activation of CaSRs may be a key to reduce the discomfort. However, such amount is corresponding to at least the double of the usual intake of calcium, and is out of the concept of PMs. Low amounts of products and in combination are sufficient to obtain a clinical activity and avoid the occurrence of side effects. Another limitation could be the intake of ascorbic acid that was much higher with SFs since its content in the red orange juice is about 75 mg in 150 mL, and with two dosages, the quantity will be about 150 mg.

However, ascorbic acid is not active in quenching peroxy radicals and hydroxyl radicals [21], which are much more affected by astaxanthin, flavonoids and lycopene. The discussion about the impact of different free radicals/oxidant on diseases is out of the scope of the present study. However, the increase of lipid peroxidation (peroxyl and hydroxyl radicals) in dysmenorrhea has been already described [22]. The present study indicates that it is possible to draw some indication in relation to the use of PMs.

In previous studies using the same PMs at the same dosages, the effect on dysmenorrhea discomfort was evident [23] and consistent with the theory that the protection of GABA_4 receptors and activation of CaSRs has some validity. For the first time it has been shown that, it is possible to control symptoms of dysmenorrhea with some foods, provided they contain specific antioxidants at the same dosage taken with capsules. The effect following the dosage of one capsule was not very consistent, and seems not different from what can be achieved with one SFs, meaning that the quantities of PMs made available with the treatments are determinant for the activity. The effect obtained with two dosages (capsules or SFs) was more evident. In these conditions, comparing the effects a significant difference was found only at one hours and four hours after the treatments (p < 0.05), while at two hours the improvement of the discomfort was very close (respectively 15 and 12 women; Chi square p > 0.05). The difference between capsules and SFs most probably belongs to the availability of the PMs, which is more rapid with capsules than with food. Food may generate a sort of slow release of the PMs; therefore, it takes more time to obtain a clinical effect. The start and duration of the effect are important points, because they give the possibility to accomplish many of the daily leaving activities.

The indirect index of activity of capsules and SFs was confirmed by the request of painkillers after the sessions, which was lower after one capsule compared to one SFs (Chi square p < 0.05). However, this difference was not evident using the double doses (p > 0.05).

In relation to foods, the indication may be that at the start of the discomfort, the intake of 16 g of salmon, 18 g of parmisan cheese, one slice of tomato with some oil, and two glasses of red orange juice are sufficient to reduce the discomfort. This was shown in 60 % of the women, although the effect was evident after two hours and lasted less than four hours more. In the same women, the use of two capsules was effective in 75 % of the cases, higher than following two SFs but not statistically significant (p > 0.05). A larger sample would have been necessary to determine a significant cut of although it is evident that the effect of capsules was more rapid and lasted longer than following SFs. One may not forget that sometimes the food repulsion is one of the symptoms of the acute phase of dysmenorrhea, and the use of capsules is much easier than the food intake.

In relation to the placebo effect, it is known that about 30 % of the cases can improve [24] and 2 or 3 cycles of treatment cycles should be given before classifying women as responders. In the present experience the capsules were administered also as fourth treatment, when the placebo effect should be in < 10 % of the cases, therefore the activity of PMs is evident in least in 65 % of the women. In relation to the antioxidant activity (PAT), the differences between capsules and SFs were not shown (p<0.05), although the values after the capsule’s intake were slightly higher, most probably due to a better bioavailability of PMs. Unfortunately, to avoid the unease due to many tip fingers, the values were limited to two hours after the intake, and values at one and four hours would have given more precise indications. During the metabolic processes in the gut, foods generate oxidation which can be partially quenched by the antioxidants available and limiting their transfer into the circulation. This is not the cases of the capsules that do not contain calories, and the PAT can detected this difference, although was not statistically significant due to the large coefficient of variation of the test.
The normal levels of PAT are between 1800 and 2400 U. Cor. [11,12], and in the present experience the average values were in the lowest range. They improved after the treatments with PMs (Table 6), and this can have an impact in the reduction of the discomfort. The differences of at least 200 U.Cor. Compared to the baseline value are necessary to be confident about the increase (or decrease). The increase of at least 200 U.Cor. Was detected respectively in 15 cases ad 12 cases after two capsules and two SFs, and was in parallel with the clinical improvement. The PFs was much less effective (three cases only) and they were not relative to the same women who showed clinical improvement. In other terms the increase produced by PFs had only a minimal effect on the dysmenorrhea (2 cases only), which seems not bound to the antioxidant level increase. This is further example that effect of PMs should be specific for the disease and a generic antioxidant activity is not sufficient.

The study shows that it is possible that SFs became part the diet to be implemented in the period of the OC suspension. They may have the same effect also without the use of any contraceptive and could be used when the symptoms appear, at any moment during of the menses since the impact on the calories intake is very low. However, this hypothesis should be validated with appropriate studies.

In two cases, only some activity on the discomfort was shown following Placebo. This may be a consequence of the environment, which was very quiet and pleasant (this was the women’s judgement), but this indicates also that in most of the women is not sufficient to counteract the daily discomfort. The use of specific PMs has been tested for the treatment of iron deficiency anemia [25], to quench the OS in-patient under treatment with levothyroxine [26], oral contraceptives [27], or suffering from Alzheimer’s disease [28], and the clinical benefit were evident showing that OS in different diseases cannot be treated with every antioxidant.

Conclusions
The protection of GABA action receptors and CaSRs with specific PMs at low dosages and in combination (bioflavonoids, lycopene, astaxanthin, and calcium) was reducing the dysmenorrhea discomfort. PMs can be given as SFs or capsules. However, the effect of one SFs or one capsule was minimal, and two capsules of two SFs were needed to reduce significantly the symptoms. Capsules showed more rapid and longer effects.

Acknowledgement
We are grateful to Prof Matrino Recchia (Statmed-Milan-Italy) for the statistical evaluation. Modigal S. deR.L de C.V Mexico City was giving the product in capsules and placebo free of charge. A patent filed by a Mexican Company protects the combination of the physiological modulators.

References
of overweight and obesity with polyglicosamine randomized study compared with placebo in subjects with caloric restriction. Current Dev Nutr. 2017; 1.


