

Research Article



Effects of D-002, a Mixture of Beeswax Alcohols, Co-Administered with Green-Lipped Mussel Extract, on Osteoarthritis Symptoms

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ABSTRACT

Osteoarthritis (OA), a degenerative joint disease affecting the cartilage and surrounding tissues, causes pain, reduces mobility, and deteriorates quality of life in adults, mainly the elderly. Most treatments for OA, such as non-steroidal anti-inflammatory drugs and analgesics, induce adverse side effects, supporting the search for effective, but safer treatments. Green-lipped mussel (*Perna canaliculus*) extracts (GME) have been useful for managing OA. D-002, a mixture of beeswax alcohols, has been shown to ameliorate experimental OA and to reduce OA symptoms. This study investigated whether administering D-002 together with GME may improve the efficacy of GME on OA symptoms. Subjects with OA symptoms were double-blindly randomized to GME capsules (50 mg of the extract/capsule) + placebo or D-002 (50 mg) tablets for 6 weeks. Symptoms were assessed by the Western Ontario and McMaster Individual Osteoarthritis Index (WOMAC) and the visual analogy scale (VAS) scores. The primary outcome was the significant reduction of total WOMAC score versus GME + placebo. WOMAC pain, joint stiffness and physical function scores, VAS score and use of rescue medications were secondary outcomes. All randomized participants (50) completed the study. Both treatments reduced significantly the total WOMAC score, pain and physical function WOMAC sub-scores, and VAS score versus baseline, but the values achieved with GME + D-002 were lower than with GME + placebo, so that GME + D-002 decreased significantly the total WOMAC score, pain and physical function WOMAC sub-scores, and VAS as compared to GME + placebo. Only one subject (treated with GME + placebo) consumed rescue medications. Treatments were well tolerated. This study demonstrates that D-002 (50 mg/day) co-administered with GME for 6 weeks, improved GME efficacy for relieving arthritic symptoms and was well tolerated.

Keywords: Anti-inflammatory, Beeswax alcohols, D-002, Green-lipped Mussel, Osteoarthritis, *Perna canaliculus*, VAS score, WOMAC score.

INTRODUCTION

Osteoarthritis (OA), an age-related degenerative joint disease involving the cartilage and surrounding tissues, is the leading cause of pain and disability in adults worldwide, mainly in the elderly. In particular, hip, knee and mixed OA are among the leading causes of worldwide disability, and this situation should increase in accordance to the increasing life expectancy and obesity epidemics of the world's population.^{1,2}

Non-pharmacologic interventions are the cornerstone of OA management,³ but current guidelines also recommend the use of analgesics, such as acetaminophen/paracetamol (first line) and non-steroidal anti-inflammatory drugs (NSAIDs) (second line) to provide symptom relief, despite they do not solve the underlying causal pathological process. Optimal treatment should combine non-pharmacological and pharmacological modalities.^{4,5} Also, the use of glucosamine/chondroitin remains controversial, and sadly in spite of promising experimental data, no disease-modifying OA drug (DMOAD) has been approved for OA management.^{6,7}

Despite their leading place to treat pain and inflammation, traditional NSAIDs (t-NSAID) (non selective inhibitors of cyclooxygenase –COX- 1 and 2) and second-

generation NSAIDs (COX2- inhibitors) can produce relevant gastrointestinal, renal and cardiovascular adverse events (AE), generally dose-dependent.^{4,5,8,9} Basically, t-NSAIDs and COX-2 inhibitors display their anti-inflammatory effects by inhibiting the COX pathway, but such a mechanism also support gastric damage linked with COX-1 inhibition, and cardiovascular AE caused by COX2 inhibition. NSAIDs-induced gastric damage results from the curtailed production of gastro protective prostaglandins and the displacement of the arachidonic acid metabolism towards the lipoxygenase (LOX) pathway.¹⁰⁻¹² This increases the synthesis of pro-inflammatory and gastro toxic leukotrienes (LT), so that specifically increased concentrations of LTB₄ and leukocytes infiltration have been found on the walls of NSAID-induced gastric ulceration,¹³ enhancing the gastro toxicity due to the prostaglandins deficit.¹⁰⁻¹²

To help minimize these risks, public health advisory have recommend that NSAIDs should be given at the lowest effective dose for the shortest duration.¹⁴ On its side, paracetamol, although safe at therapeutic doses, may cause liver toxicity and increased risk of haematological malignancies, which limit its chronic use.^{8,15,16}

This background supports the search of effective and safer treatments, like dual inhibitors of COX and 5-LOX,



which cut prostaglandins synthesis and provide anti-inflammatory effects, but prevent the switch to an increased production of LT by inhibiting 5-LOX, thus reducing the occurrence of gastric AE.¹⁷ In such regard, green-lipped mussel (*Perna canaliculus*) extracts (GME) have been shown to inhibit both COX and 5-LOX enzymes, lowering the production of LTB₄.¹⁸⁻²¹ Also, GME has been effective for alleviating OA symptoms without causing the adverse effects of NSAIDs, so that it may be an adjuvant therapy in the management of OA.²²⁻²⁶

D-002 is a mixture of six high molecular weight aliphatic alcohols (C₂₄, C₂₆, C₂₈, C₃₀, C₃₂, C₃₄) purified from beeswax²⁷ that inhibits both COX and 5-LOX activities.²⁸⁻²⁹ Consequently, D-002 has been shown to be effective in experimental models of acute and chronic inflammation³⁰⁻³³ and in experimental models of OA, wherein it has shown to protect against cartilage degeneration as well.^{34,35} Also, oral administration of D-002 (50 mg/day) for short-term (6-8 weeks) has demonstrated to reduce OA symptoms.^{36,37}

In light of these issues this study was undertaken to investigate whether administering D-002 together with GME may improve the efficacy of GME on OA symptoms.

Methods

Study design

This randomized, double-blind, placebo-controlled study was approved by the Institutional Ethics Committee of the Surgical Research Centre (Havana, Cuba). The trial was conducted according to the ethical standards established in the Declaration of Helsinki.

At enrolment oral and written explanations about the nature of the trial and the study treatments were informed to the participants by using a clear language. All participants then provided their informed written consent for participating in the trial.

Eligible subjects were randomised to GME capsules (50 mg of the extract/capsule) + D-002 (50 mg) or placebo tablets to be consumed for 6 weeks as explained below. Thereafter, subjects underwent to visits every week. Physical examinations and symptom assessment were done at each visit. Treatment compliance, control of rescue analgesic consumption and adverse experiences (AE) were controlled weekly. Laboratory examinations were done at baseline and after 3 and 6 weeks on treatment.

Study participants

Women and men of 20 - 80 years of age, with a prior diagnosis of OA of the knee, hip or fingers supported by clinical and radiological criteria, were recruited for the study. Participants were required to have a diagnosis of functional class I, II or III (mild to moderate) according to the American College of Rheumatology Criteria (ACRC).^{38,39} and a Western Ontario and McMaster Individual Osteoarthritis Index (WOMAC) \geq 30.⁴⁰⁻⁴²

Participants who suffered other forms of arthritis, arthroscopy within the past year, intra-articular injection of steroids within the past 3 months were excluded from the study. Other exclusion criteria were uncontrolled hypertension (diastolic pressure \geq 120 Hg mm), diabetes (fasting glucose $>$ 7 mmol/L), active liver or renal disease, malignancies, hospitalizations during the 6 months prior to the commencement of the trial, or to show the following laboratory abnormalities: alanine -ALT- and/or aspartate -AST- amino transferase $>$ 45 U/L, creatinine $>$ 130 μ mol/L. Also, women who were pregnant, nursing, or not taking adequate contraceptive measures were excluded from the trial.

Predefined premature discontinuations included unwillingness to follow-up, AE supporting such decision and protocol violations (failure of treatment intake \geq 5 days).

Treatment

Study treatments, produced under Licensees and good manufacturing practices conditions, came from the manufacturers (Plants of Natural Products, Laboratories MedSol, Havana, Cuba). Lyprinol® (150 mg/capsule) contained 50 mg of patented CO₂ extracted green-shell mussel non-polar lipids (PCSO-524™), 99.85 mg of pharmaceutical-grade olive oil and 0.15 mg natural vitamin E as a stabilizer. Placebo had similar composition to D-002 tablets, except the active ingredient that was replaced by lactose. Placebo and D-002 tablets were indistinguishable. GME capsules were given in their original package, placebo and D-002 in identical flasks.

At visit 2, GME capsules and coded and packaged tablets of D-002 or placebo tablets were given to study participants. Two GME capsules and D-002 or placebo tablets should be taken once daily with the breakfast for 6 weeks. The chosen dose of D-002 has been effective in previous studies in OA subjects,^{36,37} while the dosage of GME followed that recommended by the manufacturer.

The randomisation code of D-002 versus placebo was computer-generated with a fixed, not stratified randomisation method, using balanced blocks and allocation ratio of 1:1. The entire code was kept confidential at the generating place, but sealed individual coded envelopes were kept at this place and at the site of the Principal Investigator, which should be opened prematurely in case of a serious adverse event (SAE), a situation that did not happen in the trial.

Treatment compliance was controlled by counting the remainder tablets and by interviewing the subjects. At trial completion, non-used tablets were recovered. Compliance was defined as good if the subjects consumed at least 85% of the treatment scheduled from the previous visit.

Consumption of NSAIDs, steroids, cartilage or calcium supplements, or any other agent that may affect the study outcomes, except the rescue medications to treat



persistent pain: acetaminophen (maximum 2 g/day) or metamizole (maximum 600 mg/day) was not allowed during the study. All subjects were instructed to keep a diary of their consumption of rescue medications and report them at their next visit. The number of consumed rescue medication tablets was recorded at each visit.

Outcome Measures

The WOMAC questionnaire provides a validated assessment of the patient's functional capacity, specifically joint pain, stiffness and functional impairment.⁴⁰⁻⁴² The primary end-point was then to obtain a significant decrease of the total WOMAC index (Table 1) of at least 10% as compared to GME + placebo.

Table 1: Modified WOMAC Questionnaire

WOMAC pain assessment at
Walking, Stair climbing, Night, Rest, Weight bearing
WOMAC Stiffness assessment
In morning, Occurring during the day
WOMAC Physical function assessment (difficulty for)
Rising from sitting, Standing, Bending to the floor, Walking on flat, Getting in/out of a car, Going shopping, Putting on socks, Rising from bed, Taking off socks, Lying in bed, Getting in/out of bath, Sitting, Getting on/off toilet, Heavy domestic duties, Light domestic duties.

Responses of subjects corresponded to the following score: 0 = none; 1 = slight; 2 = moderate; 3 = severe; 4 = extreme

At each visit, subjects completed the WOMAC questionnaire, which includes three sections: one assess pain intensity (5 questions), other joint stiffness (2 questions), and the third one the physical function (17 questions). Individual responses were scored as 0 (none), 1 (slight), 2 (moderate), 3 (severe) and 4 (extreme). The total score ranges from 0 (the best) to 96 (the worst).

Reductions in pain, stiffness and physical function WOMAC scores, and in the Visual analogy scale (VAS) score for assessing specifically the pain^{43,44} were secondary outcomes. The VAS-visual analog scale score used a 100 mm linear measure of pain status with 0 representing no pain and 100 the worst experienced pain. Patients marked on the linear scale the amount of pain they were experiencing, and the values were recorded.

To be effective, the treatment with GME + D-002 should reduce significantly the scores as compared to GME + placebo. In order to avoid biases, subjects answered to the WOMAC and VAS questionnaires in the doctor's office before their examination.

Decrease in the use of rescue medications in the GME + placebo group versus GME + placebo was another secondary outcome. The amount of rescue medication was assessed in terms of total use at study completion.

Safety and tolerability assessment

The safety indicators included vital signs (body weight, pulse rate, blood diastolic and systolic pressure), and blood indicators (alanine amino transferase –ALT-, aspartate amino transferase –AST-, serum fasting glucose and creatinine). Blood biochemical safety indicators were assessed with enzymatic methods by using reagent kits (Roche, Switzerland) and performed in the Hitachi 709 autoanalyser (Tokyo, Japan), at the clinical laboratory of the Surgical and Medical Research Centre (Havana, Cuba). Controls of the precision and accuracy of the methods were performed.

We considered as AEs all undesirable events that occurred to a subject during the study, disregarding the cause, whenever they newly appeared during the trial. Subjects were queried by investigators for any AEs between study visits. AEs were recorded in the case record forms, including their characteristics, dates of onset and disappearance, treatments adopted and responses achieved. Severity of AEs was classified as mild, moderate or serious (SAE), mild being those that not required suspension of study medications and/or specific treatment, moderate those that caused discomfort enough and required stopping therapy and/or specific treatment, and SAE those disabling events that led to hospitalisation and/or deaths, if happened. AEs that occurred within 30 days of consuming the last study doses, monitored by direct contact with the subjects, were included in this analysis. The causal relationships between AEs and the treatments were classified by using the Naranjo algorithm.⁴⁵

Statistical Analysis

Data were analysed as per the intention to treat (ITT) approach. So, data of all randomized subjects were included in all analyses. The sample size estimation assumed a difference of $\geq 30\%$ between the reduction of the total WOMAC score from baseline with GME + D-002 and GME + placebo at study completion. Then, 25 subjects per treatment arm would be sufficient to detect such difference with 80% power and $\alpha = 0.05$. Assuming a permissible dropout rate of 10%, 55 subjects were enrolled.

Continuous data were analysed by using the Mann Whitney U test (comparisons between groups) and Wilcoxon test for paired samples (within group comparisons) with Bonferroni adjustment for multiple comparisons. Categorical variables were compared with the Fisher Exact Probability test. All tests for differences were two-tailed. Statistical analyses were conducted by using the Statistics software for Windows (USA) and MS Excel. Statistical significance was taken at the 95% level ($p < 0.05$).



RESULTS**Baseline characteristics**

Fifty-five (55) subjects were enrolled in the study. Of them, 50 were eligible for randomization. Five recruited individuals were not randomized to the active treatment because they had elevated rheumatoid arthritis (1 subject); and elevated values of fasting glucose (2 participants), creatinine (1) and transaminases (1), all above pre-defined exclusion criteria. All randomized patients (100%) completed the trial.

Baseline characteristics of study groups were well matched, so that randomization was adequate (Table 2).

Gender was predominantly female (37 women, 13 men). Co-morbidities were frequent among study subjects: 30 were hypertensive (60%), 26 (52%) had an increased body mass index (BMI) ratio (25 overweight, 1 obese), 17 had high cholesterol levels (34.0%) and 10 were diabetics (20%). Many participants (36, 72%) declared to have a sedentary life, only 5 (10%) were smokers and most (49/50, 98%) consumed some concomitant therapy.

Table 2: Baseline characteristics of study population

	GME + D-002 (n = 25)		GME + placebo (n = 25)		Total (n= 50)	
Age (years) (X±DE)	71 ± 7		69 ± 9		70 ± 8	
Body mass index (kg/m ²) (Mean ± SD)	24.9 ± 2.5		24.8 ± 2.6		24.9 ± 2.5	
Total WOMAC scores (Mean ± SD)	40.3 ± 1.7		39.8 ± 1.5		40.0 ± 1.1	
Degree of OA according to ACRC						
I	0		1		1	
II	15		16		31	
III	10		8		18	
OA diagnosis						
Mixed (knee + hip+ hands and fingers)	25		25		50	
	n	%	n	%	n	%
Sex: Female	19	76.0	18	72.0	37	74.0
Male	6	24.0	7	28.0	13	26.0
Main co-morbidities						
Hypertension	14	56.0	16	64.0	30	60.0
Overweight (kg/m ² ≥ 25, < 30)	14	56.0	11	44.0	25	50.0
Hypercholesterolemia	10	40.0	7	28.0	17	34.0
Diabetes mellitus	6	24.0	4	16.0	10	20.0
Thyroid dysfunction	1	4.0	1	4.0	2	4.0
Obesity (kg/m ² ≥ 30)	0	0.0	1	4.0	1	2.0
Lifestyle factors						
Sedentary life	19	76.0	21	84.0	36	72.0
Smoking	2	8.0	3	12.0	5	10.0
Concomitant therapy^a						
Consumption of ≥ one concomitant drug	24	96.0	25	100.0	49	98.0
Diuretics	11	44.0	10	40.0	21	42.0
Angiotensin converting enzyme inhibitors	6	24.0	8	32.0	14	28.0
Cholesterol-lowering drugs	7	28.0	8	32.0	15	30.0
Antiplatelet drugs	7	28.0	7	28.0	14	28.0
β-blockers	3	12.0	4	16.0	7	14.0
Oral hypoglycemic drugs	4	16.0	2	8.0	6	12.0
Muscle relaxants	2	8.0	2	8.0	4	8.0
Antidepressants	3	12.0	0	0.0	3	6.0
Previous diagnosis of OA	19	76.0	14	56.0	33	66.0

GME Green-lipped mussel extract, SD standard deviation, WOMAC Western Ontario and McMaster Individual Osteoarthritis Index, OA osteoarthritis, ACRC American College of Rheumatology Criteria; ^a The table includes only those concomitant drugs consumed by ≥4 subjects, No significant differences were found, (t test for independent samples for continuous variables, Fisher Exact Probability test for categorical variables)



Efficacy analysis

Treatment compliance was very good and similar in both groups. At baseline the total WOMAC scores of the two groups were statistically similar (Table 3). Treatment with GME + placebo reduced significantly ($p < 0.0001$) the total WOMAC score from the first week, and such effect did not wear off, but was enhanced over the 6 week period, so that at study completion total WOMAC scores had been lowered ($p < 0.0001$) by 79.1% as compared to baseline. The addition of D-002 to the treatment scheme enhanced the efficacy, since at study completion, not before, it decreased significantly ($p < 0.0001$) the total WOMAC score versus baseline GME + placebo (89.1% reduction versus baseline, 10% versus GME + placebo).

Table 3: Effects on the total Western Ontario and McMaster Individual Osteoarthritis Index (WOMAC) scores

Week	WOMAC Index scores §§	
	GME + D-002	GME + placebo
0 (baseline)	30.3 ± 1.2	29.6 ± 0.9
1	18.3 ± 1.5*	18.8 ± 1.0*
2	13.7 ± 1.9*	13.5 ± 1.1*
3	11.1 ± 1.8*	9.2 ± 1.3*
4	6.2 ± 1.4*	7.9 ± 1.2*
5	4.1 ± 0.9*	7.0 ± 1.1*
6	3.3 ± 1.0* ⁺	6.2 ± 1.0*

GME Green-lipped mussel extract, Values are means ± SME (standard mean error); §§ Divided into three domains: pain, stiffness and physical function; Each domain has several items. Each item is scored in a scale of 0 (none) to 4 (extreme), the lowest was the better, the highest the worst. There were a total of 24 items in the total WOMAC score; * $p < 0.0001$ Comparison versus baseline (Wilcoxon Test) (Bonferroni adjustment); + $p < 0.05$; Comparison versus GME + placebo group (Mann Whitney U test).

Table 4 summarizes the effects on pain, stiffness and physical function WOMAC scores, which were comparable in both groups at randomization. The pain score was significantly reduced with GME + placebo from the first week ($p < 0.0001$ versus baseline) and such effect progressively increased over the trial. The sequential changes in WOMAC stiffness and physical function scores were similar to that of pain WOMAC scores. Likewise, treatment with GME + D-002 significantly lowered pain, stiffness and physical function WOMAC scores in a manner that the response was also enhanced over the trial. Comparisons of final values, not of interim data, showed that GME + D-002 reduced the WOMAC pain and physical function scores more ($p < 0.05$) than GME + placebo. No significant between group differences of stiffness WOMAC scores, however, were found.

Table 5 shows the effects on the mean VAS score. Both groups exhibited similar values at randomization. Treatment with GME + placebo or with GME + D-002 lowered significantly VAS score from the first week and such responses were progressively enhanced during the

trial in both groups, so that final decreases of 82.6% (GME + placebo) and 92.8% (GME + D-002) versus baseline were seen at week 6. Final VAS scores in the group treated with GME + D-002 were significantly lower ($p < 0.001$) than in that treated with GME + placebo.

Only one participant from the GME + placebo group consumed some rescue medication (acetaminophen) during the trial, so that the consumption rate of analgesics was similar in both groups.

Safety and tolerability

Treatment was well tolerated. No subject withdrew from the trial and only one, treated with GME + placebo, experienced an AE (pneumonia) that was moderated in intensity.

Vital signs and blood parameters did not change significantly during the trial, and individual values remained within normal ranges (data not shown for simplicity).

DISCUSSION

The present study demonstrates, for the first time, that administration of GME + D-002 for 6 weeks improved the total WOMAC scores (primary efficacy outcome), as well as the pain, stiffness and physical function WOMAC scores and the VAS score for pain (secondary efficacy outcomes) more than GME + placebo. The benefit of the combined intake of GME + D-002 over GME + placebo was significant after 6 weeks on treatment, not before.

The two study groups were homogeneous at baseline, which indicates that the randomization process was adequate and that the effects here seen should be ascribed to the addition of D-002 to the conventional GME therapy, and not to a baseline difference between the groups. The mean age of study participants (70 years) is coherent with the predominant occurrence of OA among the elderly.^{1,2} In turn, the frequency of women (74.0%), was higher than that of men (26%), which agrees with a higher prevalence of OA in post-menopausal women,⁴⁶ represented by 34/37 (91.9%) randomized women. Despite the BMI ratio of randomized subjects (24.8 and 24.9 in GME + placebo and GME + D-002 groups, respectively) was below the cut-off limit to be considered as overweight (25), the frequency of participants with BMI values belonging to the overweight plus obese categories (26/50, 52%) was consistent with some of the characteristic features of OA epidemiology.⁴⁷

The high frequency ($\geq 20\%$) of hypertension (60%), overweight plus obesity (52%), hypercholesterolemia (34%), and diabetes (20.0%) among study subjects, reflects the common occurrence of concomitant coronary risk factors in Cubans of this age,⁴⁸ and are coherent with reports of co-morbid conditions in subjects with OA.^{49,50}

The present data support that GME monotherapy (here represented by the GME + placebo) is effective for symptom relief in OA, in agreement with its usefulness as



an adjunctive treatment in OA,²²⁻²⁶ since it significantly decreased all WOMAC (total, pain, stiffness and physical function) scores from the first week on treatment, such response being enhanced thereafter. Nevertheless, we should acknowledge that the efficacy of GME seen in this trial was greater than that reported in other studies. Our study found, by the first time, that GME improves OA symptoms as soon as 1 week after on therapy, since other reports account for improvements after 4-12 weeks on therapy.²²⁻²⁶ In addition, the reductions of all scores

achieved with GME + placebo at the end of the trial were marked ($\geq 70\%$), so that the total, pain, stiffness and physical WOMAC scores decreased by 79.1%, 73.6%, 84.0% and 82.0% versus baseline, when reduction of the self-evaluated VAS score was of 82.6% versus baseline. In such regard, despite the pain reduction with GME + placebo versus baseline evaluated with VAS scale was more pronounced than that assessed with WOMAC score, the differences may be considered as acceptably similar.

Table 4: Effects on pain, stiffness and physical function WOMAC scores

Week	Pain score §		Stiffness score §		Physical function §	
	GME + D-002	GME + placebo	GME + D-002	GME + placebo	GME + D-002	GME + placebo
0 (baseline)	10.4 ± 0.4	10.6 ± 0.6	2.5 ± 0.3	2.5 ± 0.3	17.4 ± 1.1	16.7 ± 0.6
1	6.2 ± 0.3**	6.6 ± 0.4**	1.3 ± 0.2*	0.9 ± 0.2*	10.8 ± 1.2**	11.3 ± 0.7**
2	4.3 ± 0.4**	4.6 ± 0.2**	0.8 ± 0.2*	0.6 ± 0.2**	8.7 ± 1.4**	8.3 ± 1.0**
3	4.0 ± 0.5**	3.9 ± 0.4**	0.8 ± 0.2*	0.4 ± 0.1*	6.3 ± 1.5**	4.9 ± 1.1**
4	3.3 ± 0.4**	3.2 ± 0.5**	0.4 ± 0.1**	0.2 ± 0.1**	2.5 ± 1.3**	4.5 ± 0.9**
5	2.2 ± 0.3**	2.7 ± 0.3**	0.4 ± 0.1**	0.3 ± 0.1**	1.6 ± 0.8**	4.0 ± 0.9**
6	1.6 ± 0.3** ⁺	2.8 ± 0.3**	0.2 ± 0.1**	0.4 ± 0.1**	1.5 ± 0.9** ⁺	3.0 ± 0.8**

GME Green-lipped mussel extract, Values are means ± SME (standard mean error); § Measured on the following scale (0-4, where 0 = none, 1 = slight, 2 = moderate, 3 = severe, 4 = extreme). The lowest the better, the highest the worst; * $p < 0.0083$, ** $p < 0.0001$, Comparisons versus baseline (Wilcoxon test) (Bonferroni adjustment); ⁺ $p < 0.05$; Comparisons versus GME + placebo group (Mann Whitney U Test)

Table 5: Effects on VAS scores §

Week	VAS scores §	
	GME + D-002	GME + placebo
0 (baseline)	61.4 ± 3.2	62.2 ± 3.6
1	51.8 ± 2.4*	50.6 ± 2.7*
2	42.4 ± 1.9**	38.6 ± 2.2**
3	33.6 ± 2.4**	32.2 ± 1.5**
4	21.2 ± 1.6**	24.8 ± 1.3**
5	10.0 ± 1.1** ⁺	15.6 ± 1.3**
6	4.4 ± 0.8** ⁺⁺	10.8 ± 0.9**

GME Green-lipped mussel extract, Values are means ± ESM; § Measured on a 100 mm scale of 0 to 100, where 0 = no pain and 100 was the worst possible pain; * $p < 0.0083$, ** $p < 0.001$; *** $p < 0.0001$, **** $p < 0.00001$ Comparisons versus baseline (Wilcoxon Test) (Bonferroni adjustment); ⁺ $p < 0.01$; ⁺⁺ $p < 0.001$; ⁺⁺⁺ $p < 0.0001$ Comparisons versus GME + placebo group (Mann Whitney U Test).

The marked and fast efficacy of GME manifested on this trial undoubtedly affected the results, mainly the sample size calculation. We assumed to find a between group difference of about 30% in the total WOMAC score achieved at the end of the study, but this difference turned to be impossible to reach since the reduction reached with GME + placebo was 79.1%, so that the maximal enhancement of the response should be < 25%. Indeed, the net reduction due to the addition of D-002 to the scheme treatment was of 10%.

This fact could also influence the time where the benefits of adding D-002 were seen, so that its benefits on all the

scores were significant at the end of the trial, not before. Based in previous data, we did not expect a significant symptom reduction with GME + placebo from the first week on treatment. Bearing in mind that D-002 has been shown to produce significant and clinically relevant reductions of WOMAC and VAS scores (> 50%) from the first week of therapy,^{36,37} we believed that D-002 could provide some benefit before the end of the study. Nevertheless, the high efficacy of GME + placebo treatment from the first week makes difficult to find a fast and relevant increase over such response.

We have not a conclusive explanation of why the efficacy of GME therapy was greater than that assumed. The lack of a third GME placebo + placebo group is a study limitation since a placebo effect on OA symptoms is a finding associated with in efficacy measurements based on subjective assessments.⁵¹ We believe that the recommendation of maintain a systematic physical activity (walking 30 minutes every day) to all trial participants, which was well followed by them could have contribute to these results. This factor, however, was common for the two groups, so that the final benefit found should be actually attributable to the addition of D-002 to the treatment.

It should be noted that the anti-inflammatory effects of GME and D-002 share a common mechanism: the dual inhibition of COX and 5-LOX activities, with marked reductions of LTB₄.^{18-20, 28, 29} In such regard, the mechanism whereby D-002 may enhance the efficacy of GME should be other than its ability to act as a dual



COX/5-LOX inhibitor. Although this study did not pretend to elucidate the mechanism that support the expected benefit, we should remark that D-002 improves all aspects of joint damage in OA models, like cartilage degeneration, pannus formation, and inflammation,^{34,35} and not just the inflammation. This chondroprotective effect of D-002 may support that the benefit derived from such action requires more time to become evident clinically than an anti-inflammatory/analgesic effect. So, despite the magnitude of the benefit was small as compared to our initial assumption, it could be relevant for the progression of the disease, an issue to be explored in longer clinical studies.

Treatments were well tolerated, and the absence of gastrointestinal AE matches well with the gastro protective effects of both GME and D-002, instead of the common gastro toxicity associated to NSAIDs use.^{23,24,52-57}

The present data suggest that D-002 treatment (50 mg/day) could be useful as an adjunctive to GME for managing OA symptoms, but this appreciation is preliminary. Since OA-related pain and impaired mobility affect the life of the sufferers, and non-selective NSAIDs, COX-2 inhibitors and analgesics induce several AE, the present data just support to develop further clinical research to demonstrate the short- and long-term effects of the combined therapy with GME + D-002 on OA symptoms.

CONCLUSION

The present results indicate that D-002 (50 mg/day) co-administered with GME for 6 weeks improved GME efficacy for relieving arthritic symptoms and was well tolerated. Then, addition of D002 to GME therapy seems to be more beneficial for managing OA symptoms than consumption of GME alone but this matter requires further experimental and clinical investigation.

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