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# Effects of D-002, a mixture of beeswax alcohols, on osteoarthritis symptoms: a randomized placebo-controlled study

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*A concise title: D-002 on osteoarthritis symptoms*
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***Abstract***

***Background and purpose: Non selective non-steroidal anti-inflammatory drugs and ciclooxygenase 2 inhibitors provide symptoms relief in osteoarthritis (OA), but produce several adverse effects (AE), which supports the search for other options. D-002, a mixture of higher aliphatic beeswax alcohols, has been shown to produce anti-inflammatory effects experimentally. This double-blind study investigated the effects of D-002 (50 mg/day) on patients with OA symptoms randomized to D-002 (50 mg) or placebo for 8 weeks.***

***Methods: The primary efficacy outcome was the reduction of the total Western Ontario and McMaster Individual Osteoarthritis Index (WOMAC) score. The decrease* on *pain, joint stiffness, physical activity scores, and use of rescue medications were secondary outcomes. Treatment tolerability was assessed.***

***Results: Four patients (3 D-002, 1 placebo) discontinued the study, none due to adverse experiences (AE). At study completion D-002 significantly reduced the total WOMAC score versus baseline (p<0.0001, 49.9%) and placebo (p <0.001, 38.0%), and also decreased the pain (p <0.001; 30.6%), joint stiffness (p <0.05 26.5%) and physical activity (p <0.0001, 41.2%) scores versus placebo. After 2 weeks on therapy all benefits were significant already. The use of rescue medication in D-002 (3/30, 10%) was lower (p <0.05) than in placebo (10/30; 33.3%). Treatment was well tolerated. Five patients (2 D-002, 3 placebo) reported mild AE.***

***Conclusions: D-002 (50 mg/day) given for 8 weeks improved OA symptoms, the benefits being evident from week 2, and was well tolerated. D-002 could be a suitable option for managing OA symptoms, but this appreciation requires extensive further research.***

***Keywords—anti-inflammatory, beeswax alcohols, D-002, osteoarthritis, WOMAC score***

## I. INTRODUCTION

Osteoarthritis (OA), a painful and disabling joint condition that affects hundreds of millions worldwide, mainly the elderly[1], requires both non-pharmacological [2, 3] and pharmacological interventions [4-7]. Current guidelines recommend use analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) to provide symptoms relief despite they do not solve the underlying causal pathological process [4-7]. Nevertheless, in view of the gastrointestinal and cardiovascular adverse effects (AE) of non selective NSAIDs and specific ciclooxygenase 2 (COX-2), respectively [8, 9], the search for better tolerated alternatives is justified.

D-002 is a mixture of six higher aliphatic primary alcohols ($C_{26}$, $C_{26}$, $C_{28}$, $C_{30}$, $C_{32}$, $C_{34}$) purified from the beeswax, wherein triacontanol ($C_{30}$) is the most abundant, with anti-inflammatory effects demonstrated in experimental models of acute and chronic inflammation [10-12]. Oral treatment with D-002, however, is gastroprotective, rather than gastrotoxic, through a mechanism that involves increased secretion and improved composition of the gastric mucus, and reduced lipid peroxidation in the gastric mucosa [14-16]. Antioxidant effects of D-002 have been demonstrated in experimental and clinical studies [21-27]. Keeping in mind this background, we believed that D-002 could improve OA symptoms.

## II. OBJECTIVE

This study investigated the effects of D-002 (50 mg/day) on patients with symptoms of OA

## III. METHODS

### 3.1 Study design

This randomized, double-blind, placebo-controlled trial was approved by the Institutional Ethics Committee of the Surgical Research Centre (Havana, Cuba) and was conducted in compliance with the ethical standards for the treatment of patients as established in the Declaration of Helsinki.

Patients were provided oral and written explanations about the nature of the trial and the study treatment in a language easily understood by the subjects in order to request their informed written consent, which was obtained from each of the participants at enrolment (visit 1).

Eligible patients were randomised to D-002 (50 mg) or placebo (visit 2) tablets, which should be taken once a day with the breakfast for 8 weeks. Subjects underwent to visits every 2 weeks (visits 3 - 6). Physical examinations and assessment of WOMAC scores were done at each visit. Treatment compliance, rescue medication (analgesics) consumption and AE were controlled from visit 3 to 6, laboratory examinations were done at baseline and every 4 weeks.

### 3.2 Study participants

Ambulatory women and men of 20 - 80 years of age, with a previous diagnosis of OA of the knee, hip or fingers supported by clinical and radiological criteria, were enrolled in the trial. Participants were required to have a diagnosis of functional class I, II or III (mild to moderate) in accordance to the American College of Rheumatology Criteria (ACRC) [28, 29].

Exclusion criteria were to suffer other forms of arthritis, any arthroscopy within the past year, intra-articular injection of steroids within the past 3 months, uncontrolled hypertension (diastolic pressure ≥ 120 mm Hg) or diabetes (fasting glucose > 7 mmol/L), active liver or renal disease, malignancies, or any other serious illnesses. Also, we excluded pregnant or lactating women, or those not taking adequate contraceptive measures and subjects with the following laboratory abnormalities: alanine -ALT- and/or aspartate amino tranferase-AST >45 U/L, creatinine > 130 µmol/L, and/or those with any hospitalization during the 6 months prior to the study.

Unwillingness to follow-up, to experience AE that justified such decision and protocol violations (failure of treatment intake ≥5 days) were predefined causes of premature discontinuations of the study.

### 3.3 Treatment

Study treatments, produced under Licensees and Good Manufacturing Practices conditions, came from the manufacturers (Plants of Natural Products, Laboratorios MedSol, Havana, Cuba). D-002 content was assessed by using a gas chromatography method [30]. Placebo had similar composition to D-002 tablets, except the active ingredient that was replaced by lactose. Treatments were packaged in PVC-aluminium sealed burbles (blisters).

At visit 2, identical coded and packaged tablets of study treatments (D-002 50 mg or placebo) were given to study subjects. Tables were taken once a day with the breakfast for 8 weeks. No dose titration was done. The randomisation code was computer-generated with a fixed, not stratified randomisation method, using balanced blocks and allocation ratio of 1:1. The dose of D-002 (50 mg/day) selected had been shown to produce effective antioxidant effects in clinical studies [24-27].

The entire code was kept confidential at the generating place. Sealed individual envelopes with codes of each subject were kept at the generating place and at the site of the Principal Investigator, which should be opened prematurely in case of occurring a serious adverse event (SAE), a situation that did not occur in the trial.

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

Subjects were not allowed to consume 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). The number of consumed rescue medication tablets was recorded at each visit.

### 3.4 Efficacy Assessment

The primary end-point was to obtain a significant reduction of the total Western Ontario and McMaster Individual Osteoarthritis Composite (WOMAC) index [31, 32] (Table 1) of not less than 30% as compared to placebo. At each visit, subjects completed the WOMAC questionnaire, which consists of three sections, one that assess pain intensity (5 questions), other joint stiffness (2 questions), and the third the physical function (17 questions). Individual responses were scored on the following scale: 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 scores were secondary efficacy variables. For efficacy, the score reductions should be significant as compared to placebo.

Use of rescue medication was addressed as a collateral measure of efficacy. The amount of rescue medication was assessed in terms of total use at the conclusion of the study period.

**3.5 Safety and tolerability assessment**

The safety indicators included vital signs (body weight, pulse rate, blood diastolic and systolic pressure), and blood indicators (erythrocyte sedimentation rate, ALT, 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), erythrocyte sedimentation rate was assessed by conventional method, all done 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 easily tolerated 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 leaded 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 [33].

**3.6 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 ≥ 30% between the reduction of WOMAC total scores from baseline with D-002 and placebo at study completion. Then, 30 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%, 65 subjects were enrolled.

Continuous data were analyzed by using the following tests: unpaired and paired t tests, Bonferroni adjustment for multiple comparisons, or ANOVA, as appropriate. Categorical variables were compared with the Fisher Exact Probability test. All statistical tests for differences were 2-tailed. The following software was used for the comparisons: Statistics software for Windows (USA) and MS Excel. Statistical significance was taken at the 95% level ($p < 0.05$).

## IV. RESULTS

**4.1 Baseline characteristics**

Sixty-five (65) subjects were enrolled in the study. Of them, 60 were eligible for randomization. Five enrolled subjects did not pass to the active treatment step because of the following reasons: fasting glucose > 7 mmol/L (2 subjects), AST and ALT values > 55 U/L (2 cases) and rheumatoid arthritis (1). Of the 60 patients (50 women, 10 men) (mean age 52 years) included, 56 completed the trial and 4 (3 D-002, 1 placebo) withdrew from the study, none due to AE.

All baseline characteristics of both groups were similar, so that subject randomization was effective (Table 2). Gender was predominantly female 50/60 (83.3%) vs. males 10/60 (16.7%). Study population included a high frequency (>30%) of some co-morbid conditions like hypertension (41.7%) and hypercholesterolemia (36.7%), and some negative lifestyle factors, like sedentary life (73.3%) and smoking (20.0%). A total of 45/60 (75%) randomized subjects consumed some concomitant therapy during the study.

Four subjects (3 D-002, 1 placebo) withdrew prematurely from the study: 2 due to protocol violations (D-002) and 2 due to unwillingness to follow-up (1 D-002, 1 placebo).

**4.2 Efficacy analysis**

Treatment compliance was very good and similar in both groups.

At baseline the total WOMAC scores (mean ± SD) in the D-002 and placebo groups were 41.5 ± 13.0 and 42.1 ± 14.9, respectively, without significant differences between the groups (Table 2). After 2 weeks of treatment, the score was significantly reduced in D-002 ($p < 0.001$ versus baseline) and placebo ($p < 0.05$ versus baseline) by 30.8 and 8.8%, respectively, but the total WOMAC score in D-002 group was lower ($p < 0.05$) than in placebo (net difference of 22% versus placebo). The treatment effect did not wear off, but even improved, during the treatment period. At study completion D-002 had reduced significantly the total WOMAC score ($p < 0.0001$ versus baseline and placebo) (49.9% versus baseline, 38.0% versus placebo)

The mean ± SD baseline WOMAC pain scores were 10.5 ± 3.5 (D-002 group) and 9.9 ± 4.0 (placebo) (Table 4). After 2 weeks of treatment, pain score was significant lower in the D-002 (28.6% reduction versus

baseline, p <0.001) and placebo (11.1% reduction versus baseline, p <0.05) groups, so that the net difference versus placebo was 17.5%. The treatment effect was enhanced over the trial, so that at the study completion pain score decreased significantly with D-002 (45.7% versus baseline, p <0.0001) 30.6%, p <0.01 versus placebo).
At baseline the mean stiffness score was 3.4 ± 2.1 in the D-002 group, and 3.4 ± 1.8 in placebo. After week 2, the score was significantly reduced with D-002 (32.3% versus baseline, p <0.001; 23.5% versus placebo, p <0.01). At study completion the reduction in stiffness with D-002 (p <0.01 versus baseline, p <0.05 versus placebo) was of 41.2% as compared to baseline, and of 26.5% versus placebo.

The sequential changes in WOMAC physical function scores were similar to those occurred with the other subset and total WOMAC scores. Meanwhile the mean baseline values of D-002 (27.5 ± 9.6) and placebo (28.7 ± 10.8) groups were similar, the scores at 2, 4 and 8 weeks of therapy in the D-002 group were significantly lower (p <0.001 for week 2, p <0.0001 for weeks 4 and 8) than in placebo. The score reductions with D-002 were successively accentuated over the 8 week period, so that the decrease of the physical function score with D-002 at trial completion was of 51.3% and 41.2% as compared to baseline and placebo, respectively.

Thirteen (13) subjects (3 D-002, 10%; 10 placebo, 33.3%) consumed acetaminophen or metamizole during the trial, so that the consumption of rescue medication in D-002 group was lower (p <0.05) than in placebo group.

**4.3 Safety and tolerability**

Vital signs and blood indicators did not change during the study, with the exception of erythrocyte sedimentation rate, which significantly lowered from baseline in D-002 group as compared to baseline (p <0.0001) and placebo (p <0.05) (data not shown for simplicity). This reduction, albeit significant, was not considered as an AE.

## V. DISCUSSION

Using a randomized, double-blind placebo-controlled design the present study demonstrated, by the first time, that D-002 improved pain, stiffness, physical function and overall activity in subjects with OA symptoms as compared to baseline and placebo. The D-002 effects were persistent over the trial, so that it produced sustained benefits, distinguishable from placebo.

D-002 and placebo groups were homogeneous at baseline, which indicates that randomization was effective, and that our results are not attributable to initial differences between the groups, but to D-002 treatment. The mean age of study subjects (52 years) falls within that expected for this disease. Most subjects (83.3%) were women, consistent with a higher prevalence of OA in women, mainly post-menopausal [34], a condition present in 31 of the 50 randomized women (62%). The high frequency of sedentary life (81.7%), hypertension (41.7%), hypercholesterolemia (36.7%) and smoking (20%) among study subjects, not only reflects an undesirable occurrence of coronary risk factors, common in Cuban subjects of this age [35], but agrees with reports of co-morbidity of OA in middle-aged and older subjects [36].

Both groups displayed an improvement of total WOMAC values over the 8 weeks of treatment, including placebo, but these reductions, however, were greater in D-002 than in placebo group. A similar picture was seen for subset WOMAC scores. The D-002 group significant reductions of the total (primary efficacy variable) and subset (secondary efficacy variables) WOMAC scores were evident from the week 2, with appreciable improvements with continued administration. At study completion pain, stiffness, physical function and total WOMAC scores decreased by 45.7%, 41.2%, 51.3% and 49.9% as compared to baseline, respectively (reductions versus placebo of 30.6%, 26.5%, 41.2%, and 38.0%, respectively).

In contrast, although reductions with placebo were also seen, they remained almost stationary over the treatment. The final decreases of pain, stiffness, physical function and total WOMAC scores in placebo were 15.1%, 14.7%, 10.1% and 11.9%, respectively, versus baseline. This improvement with placebo, however, was not totally unexpected, as it can occur in any efficacy measurement based on subjective assessment. Possible explanations for this finding could include that study subjects may have had high expectations of the benefits of study treatments, despite they were due informed about the use of placebo, and that 33.3% of placebo subjects used rescue medications over the trial, so that the reductions observed in the placebo group could be influenced by this fact, as in other placebo-controlled studies in subjects with OA [37].

We believe that the placebo effect here seen, however, does not limit the acceptance of the D-002 efficacy for alleviating OA symptoms here demonstrated. The evident significance of the score reductions with D-002 as compared to placebo and the fact that, opposite to the increasingly efficacy of D-002, the magnitude of the placebo effect was steady over the trial support this appreciation. Besides, since the use of rescue medication use in placebo was lower than in D-002 group, it becomes obvious that the efficacy results with D-002 are actually due to the treatment. In addition, this fact minimizes the possible contribution of a masking effect of rescue medication to efficacy results, since despite to be more frequently used in placebo, all reductions, even for pain subset, were more effective in D-002 group.

Although the detailed mechanisms by which D-002 achieves reductions of OA symptoms remain unclear, they should be associated with its anti-inflammatory [11, 12], and moderate analgesic effects [12]. D-002 has been shown to reduce leukotriene B4 content in the pleural exudates of rats with carrageenan-induced pleurisy [11], and myeloperoxidase activity in mice with xylene-induced ear oedema [12], which suggest that its effects on lipooxygenase (5-LOX) pathway and neutrophil infiltration, respectively, could be associated with its anti-inflammatory effects *in vivo*. Currently, the effects of D-002 on both COX and 5-LOX activities are under study.

Some nutraceuticals with anti-inflammatory effects and good gastrointestinal safety profile have shown benefits in experimental arthritis and in OA patient, as well [38]. Since COX-2 is intimately involved in OA symptoms, dietary n-3 polyunsaturated fatty acids (PUFA), which inhibit COX activity, are thought to be beneficial for managing arthritis, and given together with glucosamine sulfate, has provided a superior efficacy as compared to glucosamine alone in patients with OA [39, 40].

D-002 resulted safe and well tolerated, consistent with previous clinical studies [18-20, 24-27]. In particular, the absence of gastrointestinal AEs matches well with the gastroprotective effects of D-002 [18-20]. The existing concern about the increased risk for cardiovascular disease and stroke with COX-2 inhibitors, and the gastrointestinal and renal complications produced by non-selective NSAIDs [7-9], remains open perspectives for new therapies, including complementary medicines. Indeed, OA sufferers using NSAIDs have an increased risk of suffering AEs that require hospitalization as compared to the non-users [9]. Then, these subjects look to other alternatives for symptomatic relief devoid of relevant AEs [38].

There are a number of limitations of the current study that require further research. The number of study participants is small, and we did not confirm the previous diagnosis of OA. Then, although the reductions of all WOMAC scores with D-002 as compared to placebo suggest that this treatment, devoid of gastrotoxic effects, could be useful to manage OA symptoms, the efficacy of D-002 needs to be adequately determined in further clinical trials to support this preliminary concept.

## VI. CONCLUSION

D-002 treatment (50 mg/day) for 8 weeks improved OA symptoms as compared to placebo. The benefits were evident from week 2. Treatment tolerability was very good. Then, D-002 could be a suitable option for managing OA symptoms, but this appreciation requires extensive further research.

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**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)** |
| Descending stairs |
| Ascending stairs |
| 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 |
| While working |
| Sitting cross legged |
| While cycling |
| While driving vehicle |
| While praying |

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

**Table 2. Baseline demographic and clinical characteristics of study population**

| **Characteristic** | **D-002 (n = 30)** | | **Placebo (n = 30)** | | **Total (n = 60)** | |
|---|---|---|---|---|---|---|
| **Age (years)** | | | | | | |
| Mean ± SD | 52 ± 10 | | 53 ± 10 | | 52 ± 10 | |
| Range | 31 - 71 | | 34 - 72 | | 31 - 72 | |
| **Body mass index (kg/m$^2$) (Mean ± SD)** | 27.2 ± 4.3 | | 26.5 ± 4.4 | | 26.9 ± 4.3 | |
| **Total WOMAC scores (Mean ± SD)** | 41.5 ± 13.0 | | 42.1 ± 14.9 | | 41.8 ± 13.8 | |
| **Sex** | **n** | **%** | **n** | **%** | **n** | **%** |
| Female | 26 | 86.7 | 24 | 80.0 | 50 | 83.3 |
| Male | 4 | 13.3 | 6 | 20.0 | 10 | 16.7 |
| | | | | | | |
| **Subjects with previous NSAID therapy** | 17 | 56.7 | 15 | 50.0 | 32 | 53.3 |
| **Main concomitant conditions** | | | | | | |
| Hypertension | 12 | 40.0 | 13 | 43.3 | 25 | 41.7 |
| Hypercholesterolemia | 11 | 36.7 | 11 | 36.7 | 22 | 36.7 |
| Thyroid dysfunction | 3 | 10.0 | 3 | 10.0 | 6 | 10.0 |
| Diabetes | 1 | 3.3 | 1 | 3.3 | 2 | 3.3 |
| **Lifestyle factors** | | | | | | |
| Sedentary life | 24 | 80.0 | 25 | 83.3 | 49 | 81.7 |
| Smoking | 5 | 16.6 | 7 | 23.3 | 12 | 20.0 |
| **Concomitant therapy** [a] | | | | | | |
| Consumption of al least one concomitant | 23 | 76.7 | 22 | 73.3 | 45 | 75.0 |
| Diuretics | 9 | 30.0 | 10 | 33.3 | 19 | 31.7 |
| Cholesterol-lowering drugs | 10 | 33.3 | 9 | 30.0 | 19 | 31.7 |
| Angiotensin converting enzyme inhibitors | 6 | 20.0 | 6 | 20.0 | 12 | 20.0 |
| Thyroid hormones | 3 | 10.0 | 3 | 10.0 | 6 | 10.0 |
| β-blockers | 2 | 6.7 | 3 | 10.0 | 5 | 8.3 |
| Calcium antagonists | 1 | 3.3 | 2 | 6.7 | 3 | 5.0 |

SD: standard deviation, n: number of cases , NSAID: non steroidal anti-inflammatory drugs, OA: osteoarthritis

[a] The table includes only those consumed by ≥ 2 subjects

No significant between group differences were found, (t test for independent samples for continuous variables, Fisher Exact Probability test for categorical variables)

**Table 3. Changes in Total Western Ontario and McMaster Individual Osteoarthritis Index (WOMAC) scores**

| | **WOMAC Index scores** | |
|---|---|---|
| | **D-002** | **Placebo** |
| **Baseline** | 41.5 ± 13.0 | 42.1 ± 14.9 |
| **2 Weeks** | 28.7 ± 11.9 $^{**+}$ | 38.4 ± 15.0 $^{*}$ |
| **% change** | **- 30.8** $^{+}$ | **- 8.8** |
| **4 Weeks** | 25.0 ± 12.2 $^{**+++}$ | 38.1 ± 15.1 $^{**}$ |
| **% change** | **- 39.8** $^{+++}$ | **- 9.5** |
| **6 Weeks** | 23.6 ± 10.4 $^{***+++}$ | 38.1 ± 15.1 $^{**}$ |
| **% change** | **- 43.1** $^{+++}$ | **- 9.5** |

| | | |
|---|---|---|
| **8 Weeks** | 20.8 ± 10.2 $^{***++++}$ | 37.1 ± 14.6 $^{**}$ |
| **% change** | **- 49.9** $^{+++}$ | **- 11.9** |

Values are means ± SD, p < 0.0001 (ANOVA test)
*p < 0.01; **p < 0.001; ***p < 0.0001 versus baseline (t test for dependent samples) (Bonferroni adjustment)
$^{+}$p <0.05; $^{++}$p < 0.01; $^{+++}$p < 0.001 $^{++++}$p < 0.0001 versus placebo (t test for independent samples)

**Table 4. Changes in pain, stiffness and physical function scores by treatment group**

| | **Pain score** | | **Stiffness score** | | **Physical function** | |
|---|---|---|---|---|---|---|
| | **D-002** | **Placebo** | **D-002** | **Placebo** | **D-002** | **Placebo** |
| **Baseline** | 10.5 ± 3.5 | 9.9 ± 4.0 | 3.4 ± 2.1 | 3.4 ± 1.8 | 27.5 ± 9.6 | 28.7 ± 10.8 |
| **2 Weeks** | 7.5 ± 4.4 $^{**}$ | 8.8 ± 4.2 $^{*}$ | 2.3 ± 1.1 $^{**+}$ | 3.1 ± 1.7 | 18.9 ± 7.5 $^{**++}$ | 26.4 ±10.9 $^{*}$ |
| **% change** | **-28.6** | **-11.1** | **- 32.3 +** | **- 8.8** | **- 31.3 ++** | **- 8.0** |
| **4 Weeks** | 6.5 ± 4.5 $^{**}$ | 8.7 ± 4.2 $^{*}$ | 2.0 ± 1.1 $^{**+}$ | 3.0 ± 1.7 | 16.5 ± 7.8 $^{**+++}$ | 26.4 ± 10.9 $^{**}$ |
| **% change** | **-38.1** $^{+}$ | **-12.1** | **- 41.2** $^{+}$ | **-11.8** | **- 40.0** $^{+++}$ | **- 8.0** |
| **6 Weeks** | 6.0 ± 4.0 $^{***+}$ | 8.7 ± 4.2 $^{*}$ | 2.0 ± 1.0 $^{**+}$ | 3.0 ± 1.7 | 15.6 ± 6.8 $^{**+++}$ | 26.4 ± 10.9 $^{**}$ |
| **% change** | **- 42.8** $^{+}$ | **-12.1** | **- 41.2** $^{+}$ | **- 11.8** | **- 43.3** $^{+++}$ | **- 8.0** |
| **8 Weeks** | 5.7± 4.0 $^{***++}$ | 8.4 ± 3.6 $^{*}$ | 2.0 ± 0.9 $^{**+}$ | 2.9 ± 1.6 | 13.4 ± 6.6 $^{**+++}$ | 25.8 ± 10.7 $^{**}$ |
| **% change** | **- 45.7 +** | **-15.1** | **- 41.2 +** | **- 14.7** | **- 51.3 +++** | **- 10.1** |

Values are means ± SD, p < 0.01, p < 0.05 and p < 0.0001 for pain, stiffness and physical activity scores, respectively (ANOVA test)
*p < 0.01; **p < 0.001; ***p < 0.0001 versus baseline (t test for dependent samples) (Bonferroni adjustment)
$^{+}$p <0.05; $^{++}$p < 0.01; $^{+++}$p < 0.001 $^{++++}$p < 0.0001 versus placebo (t test for independent samples)

**Table 5. Occurrence of adverse events (AEs) during the study**

| | **D-002 (n = 30)** | | **Placebo (n = 30)** | |
|---|---|---|---|---|
| **AEs** | **n** | **%** | **n** | **%** |
| Headache | 1 | 3.3 | 1 | 3.3 |
| Somnolence | 1 | 3.3 | 0 | 0.0 |
| Cervical pain | 0 | 0.0 | 1 | 3.3 |
| Lower limb pain | 0 | 0.0 | 1 | 3.3 |
| Increased appetite | 0 | 0.0 | 1 | 3.3 |
| **Total of AEs reported** | **2** | **6.7** | **4** | **13.3** |
| **Subjects experiencing AEs** | **2** | **6.7** | **3** | **10.0** |

n: number of cases
No significant between group differences were found (Fisher Exact Probability test)