

NEM[®] Brand Eggshell Membrane in the Treatment of Pain and Stiffness Associated with Knee Osteoarthritis: An Open Label Clinical Study

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Abstract

Objective: NEM[®] Brand Eggshell Membrane contains collagen and glycosaminoglycans that have beneficial effects in the treatment of Osteoarthritis (OA). A single-center, open-label clinical study was conducted to evaluate the efficacy and safety of NEM[®] in management of pain and stiffness associated with knee OA.

Methods: Seventy subjects with knee OA received oral NEM[®] 500 mg once daily for 60 days. The primary outcome measure was to evaluate the effectiveness of NEM[®] in reducing pain and stiffness associated with knee OA. The primary endpoints were the change in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Visual Analog Scale for Pain (VAS pain) and Lequesne Algofunctional Index measured after 10, 30 and 60 days of NEM[®] supplementation.

Results: NEM[®] treatment resulted in reduction of WOMAC overall scores at 10 days (16.6%, $p=0.012$), at 30 days (31.8%, $p<0.0001$) and at 60 days (46.7%, $p<0.0001$) post-treatment compared to baseline values. VAS pain was reduced at 10 days (19.6%, $p<0.0001$), at 30 days (31.8%, $p<0.0001$) and at 60 days (49.0%, $p<0.0001$). Overall Lequesne scores were reduced at 10 days (11.2%, $p=0.0002$), at 30 days (24.0%, $p<0.0001$) and at 60 days (36.8%, $p<0.0001$). In a Global Assessment, 68.6% of patients and 78.6% physicians rated the efficacy of NEM[®] as excellent or good. Three mild and transient, and no serious adverse events were reported.

Conclusions: NEM[®] supplementation resulted in rapid and significant reduction of joint pain and stiffness (at 10 days) which were further improved at 60 days. NEM[®] treatment was safe and well tolerated.

Keywords: Osteoarthritis; Pain; Stiffness; Natural eggshell membrane (NEM); Knee; Dietary supplement; Glycosaminoglycans

Introduction

Osteoarthritis (OA) is a degenerative disease characterized by joint pain and stiffness that can cause physical dysfunction and decreased quality of life. OA is a common disease that occurs most often in people over 50 years of age, but also in younger population. The cartilage of articular joints is primarily affected in OA and the knee is one of the most commonly affected joints. The structural changes of articular cartilage, synovial membrane and subchondral bone are due to a combination of risk factors, including aging, obesity, being female, genetics and joint injury. In knee OA, the cartilage of knee joint gradually roughens, becomes thin or wears away causing bone rubbing on bone and pain. OA develops slowly and the joint pain and stiffness usually worsen as the disease progresses [1]. Inflammation is involved in the pathogenesis of OA. Synovitis is common in early and advanced OA and has been associated with knee pain and swelling and progression of cartilage degeneration. Synovium in OA becomes infiltrated by inflammatory cells and increased local levels of pro-inflammatory cytokines such as interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , and IL-6 produced by these cells can enhance cartilage degradation or induce bone resorption [2-4].

Medication-based therapies in OA comprise different drugs, including analgesics (e.g. paracetamol, hydrocodone) or non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. ibuprofen, celecoxib, etc.), alone or in combination. These therapies have shown limited effectiveness in clinical studies or may have significant and serious side effects [5-8].

NEM[®] brand eggshell membrane has shown good efficacy in relieving joint pain and stiffness in several clinical trials [9-12]. Eggshell membrane is primarily composed of collagen type I [13], but also of other bioactive components, namely glycosaminoglycans including dermatan sulfate, chondroitin sulfate, hyaluronic acid and hexosamines, such as glucosamine [14,15]. These constituents have been shown to have beneficial effects in the treatment of OA [16,17]. NEM[®] brand eggshell membrane has been shown to down-regulate various pro-inflammatory cytokines, including interleukin-1 beta (IL-1 β) and tumor necrosis factor alpha (TNF- α) both in vitro [18] and in vivo [19]. ESM Technologies, LLC (Carthage, MO, USA), has developed methods to efficiently and effectively separate eggshell membrane from eggshells. The isolated membrane is then partially hydrolyzed using a proprietary process and dry-blended to produce NEM[®] brand eggshell membrane.

Here, we report the findings of the single-center, 2-month open-label, study that was designed to evaluate the efficacy of NEM[®] in the

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Received October 08, 2019; **Accepted** December 10, 2019; **Published** December 18, 2019

Citation: Damjanov N, Novkovic S, Basaric M, Nikolic AK, Vagic K, et al. (2019) NEM[®] Brand Eggshell Membrane in the Treatment of Pain and Stiffness Associated with Knee Osteoarthritis: An Open Label Clinical Study. J Arthritis 8: 287.

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reduction joint pain, stiffness and functional disability in patients with moderate to severe knee OA and safety and tolerability of NEM® supplementation.

Patients and Methods

Study design

This study was performed according to a prospective, single-center, open-label design and was conducted at the Institute of Rheumatology, Belgrade, Serbia and conducted in accordance with the Declaration of Helsinki to ensure protection of human subjects.

The study protocol was reviewed and approved by an ethics committee at the study site. All OA patients enrolled in the study were appropriately informed about the study and signed informed consent.

The patients with knee OA were treated with oral NEM® 500 mg capsules (Pharmanova, Serbia) once daily for 60 days. NEM® capsules were stored in closed containers at ambient temperature. Patients were required to stop all current pain relief medications, except for paracetamol, for at least 15 days for NSAID and 3 months for glucocorticoids prior to enrollment. Clinic visits were scheduled at 10, 30, and 60 days following the onset of treatment. Treatment compliance was checked at clinic visits by patient interview and by counting the number of unused doses of the study medication. Paracetamol was allowed for pain relief, if necessary, up to 4 tablets (500mg) per day. Subjects recorded the time and amount of paracetamol taken in patient diaries.

Study population

The study included 70 patients with knee osteoarthritis. Inclusion criteria for participation in the study were: patients aged 45-75 years diagnosed with knee osteoarthritis [20] and with persistent knee pain of at least month duration and not associated with the recent trauma; the persistent knee pain associated with OA with a baseline score of 20 mm to 70 mm on the Patient's Assessment of Arthritis Pain-Visual Analog Scale (VAS) and patients that have been diagnosed with radiographic grades I-III of OA according to the Kellgren-Lawrence (KL) score [21].

Exclusion criteria for participation in the study were: pregnancy or breastfeeding, known allergy to eggs or egg products, hypersensitivity to any of the ingredients of NEM® capsules, rheumatic inflammatory diseases or systemic connective tissue diseases, co-morbidities including malignant, hematological, liver, kidney or metabolic diseases such as diabetes mellitus, body mass index greater than ≥ 29.9 kg/m², treatments with glucosamine, chondroitin sulfate, or methylsulfonylmethane (MSM), collagen, hyaluronic acid in the last three months, medications with NSAID in the last 15 days or corticosteroids in the last 3 months, and any other criteria which, by the investigator's opinion, would jeopardize patient's compliance with the study protocol.

Treatment response

The primary outcome measure of this study was to evaluate the effectiveness of NEM® in reducing pain and stiffness associated with OA of the knee. The primary endpoints were the change in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC-overall, pain, stiffness and function), Visual Analog Scale for Pain (VAS pain) and Lequesne Algofunctional Index measured after 10, 30 and 60 days of NEM® supplementation. Patient's and physician's estimation of disease activity were also estimated.

The WOMAC OA index is a tridimensional self-administered health

status measure of pain, stiffness and physical functional disability [22]. The "pain," "stiffness" and "function" subscales consist of five, two and seventeen items, respectively. Each of the overall 24 questions is graded on a scale ranging from "none" to "extreme" with five possible answers to every question (0=none, 1=mild, 2=moderate, 3=severe, 4=extreme). The maximum score is 20 points for pain, 8 points for stiffness and 68 points for the physical function. Higher scores indicate the presence of worse symptoms, greater limitations and poorer health. Endpoints were then compared to pretreatment assessments.

A Visual Analog Scale (VAS) for pain was used to evaluate the severity of joint pain in OA patients and ranged from 0 (no problem) to 100 mm (extreme problems). VAS is considered to be reliable and valid for the assessment of subjects with specific knee conditions [23] In addition, patient's and physician's assessment of disease activity scores were collected using a visual-analog scale.

The Lequesne OA index is a disease-specific questionnaire, which directly estimates symptoms and functions and has an interview format [24,25]. Lequesne questionnaire includes three sections with a total of 11 questions: pain or discomfort (5 items), maximum distance walked (2 items) and activities of daily living (4 items). The sum of all questions is the overall Lequesne OA index score. Each section has a score ranging from 0 to 8, resulting in a total score between 0 and 24. Higher scores indicate a worse health condition. A sum between 1 and 4 denotes a mild disability, 5-7 moderate, 8-10 severe, 11-13 very severe and greater than or equal to 14 extremely severe.

In a Global Assessment patients and physicians rated the efficacy and the safety and tolerability of NEM® on a scale ranging from: 1=no effect, 2= bad, 3=moderate, 4= good, 5=excellent following the 60 days of treatment.

Adverse events

Secondary objectives of the study were to evaluate tolerability and any adverse reactions associated with supplementation with NEM®. The subjects' self-assessment records were reviewed. Adverse events were assessed by the clinical investigator at each study visit.

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 i MedCalc Version 8.1 statistical programs. The internal consistency reliability of the WOMAC and Lequesne algofunctional indices was tested with Cronbach's alpha coefficient [26] which showed acceptable reliability ($\alpha=0.96$ for WOMAC and $\alpha=0.84$ for Lequesne indices). Descriptive statistics were performed to calculate the means, standard deviations, standard error of mean, medians, minimum and confidence interval where appropriate. For categorical variables, frequencies and percentages were provided. Following evaluation for normality, data determined to be parametric were evaluated by univariate analysis of variance (ANOVA). If ANOVA verified significance at $p < 0.05$, pairwise comparisons were made using a parametric test to identify statistical differences. Post-baseline statistical analyses were done as repeated measures univariate analysis of variance (RM-ANOVA) with post hoc Bonferonni analysis. Correlation between numerical variables has been done by Pearson coefficient (r). Statistical significance was accepted at $p < 0.05$.

Results

Demographic and clinical characteristics of OA patients

A total of seventy subjects between the ages of 45 and 75 with

osteoarthritis of the knee were enrolled in the study. The mean age of all enrolled subjects was 64.0 years. Of all enrolled patients 6% were between the ages of 45 and 50, 17% between 51 and 60, 54% between 61 and 70 and 23% between 71 and 75 years. Of these subjects, fifty-nine (84.3%) were female and eleven (15.7%) were male. The distribution of OA patients by age between genders was similar, with no significant difference in age between females and males. The mean body-mass Index (BMI) of all enrolled patients was 25.9, 23 patients (32.9%) had BMI 18.0-24.9, while 47 patients (67.1%) had BMI 25.0-29.9. The mean disease duration of all enrolled patients was 80.6 months with median duration of 48 months (minimum of 7 and maximum of 404 months). The majority of patients (76%) had disease duration in the range of zero to 120 months. Of the seventy patients, forty-two (60.0%) had bilateral incidence of knee OA. Of all patients, 16 had less severe disease (Kellgren-Lawrence score grade 1); while 54 patients had more severe disease (Kellgren-Lawrence score grade 2/3). Bilateral affection of knee joints was present in 9 patients with grade 1, in 18 patients with grade 2 and in 15 patients with grade 3 KL score. The mean disease duration was 36.2 months in patients with KL grade 1, 73.9 months in those with grade 2 and 122.5 months in grade 3 patients. All seventy subjects completed baseline assessments and the 2-month study per the protocol. Compliance with the study treatment regimen was good. Demographic and clinical characteristics of OA patients are presented in Table 1.

Effects of NEM® supplementation on WOMAC knee osteoarthritis indices

Analysis of the primary outcome measure revealed that supplementation with NEM® resulted in a significant treatment response from baseline at all-time points for overall WOMAC scores

| | | |
|--|--------------|-------------|
| Age (yrs), mean (SEM) | | 64.0 (0.9) |
| Gender | Male N (%) | 11 (15.7) |
| | Female N (%) | 59 (84.3) |
| Height (cm), mean (SEM) | | 167.6 (1.2) |
| Weight (kg), mean (SEM) | | 72.9 (1.3) |
| Body-mass Index (kg/m ²), mean (SEM) | | 25.9 (0.3) |
| Disease duration (months), mean (SEM) | | 80.6 (8.3) |
| Disease duration (months) | | |
| 0-120 (N, %) | | 53 (76) |
| 120-240 (N, %) | | 14 (20) |
| 240-360 (N, %) | | 2 (3) |
| 360-480 (N, %) | | 1 (1) |
| Affected joints | | |
| Left knee (N, %) | | 13 (18.6) |
| Right knee (N, %) | | 15 (21.4) |
| Bilateral (N, %) | | 42 (60.0) |
| Radiographic classification (Kellgren-Lawrence score) | | |
| Grade 1 (N, %) | | 16 (22.9) |
| Grade 2 (N, %) | | 32 (45.7) |
| Grade 3 (N, %) | | 22 (31.4) |

Table 1: Demographic and clinical characteristics of patients with knee OA.

in patients with knee OA in this study. Moreover, overall WOMAC scores significantly decreased between all-time points over the study period as shown in Table 2.

A significant decrease in overall WOMAC scores was observed after only 10 days (16.6% reduction, $p=0.012$) following NEM® treatment. After 30 days (31.8% reduction, $p<0.0001$) and 60 days (46.7% reduction, $p<0.0001$) of NEM® therapy further improvement of WOMAC scores was observed. Analysis of pain related WOMAC scores revealed significant reduction of pain from baseline at all-time points. Pain related WOMAC scores significantly decreased between the all-time points over the study period (Table 2). Reduction of pain was observed after only 10 days (19.8% reduction, $p<0.0001$), with further reductions after 30 days (32.1% reduction, $p<0.0001$) and 60 days (51.9% reduction, $p<0.0001$) of NEM® therapy.

Supplementation with NEM® resulted in significant reduction of stiffness from baseline at all-time points. Stiffness related WOMAC scores significantly decreased between all-time points over the study period (Table 2). Reduction of stiffness was observed after only 10 days (12.9% reduction, $p=0.012$), after 30 days (35.5% reduction, $p<0.0001$) and after 60 days (51.6% reduction, $p<0.0001$) of NEM® supplementation.

Physical function improved after supplementation with NEM® as revealed by significantly decreased function related WOMAC scores from baseline at all-time points. Namely, mean function WOMAC subscores showed a 16.4% absolute improvement at 10 days ($p<0.0001$), 31.6% at 30 days ($p<0.0001$) and 45.1% at 60 days ($p<0.0001$). Also, significant improvement in function was observed between all study points over the study period as shown in Table 2.

Next, we analyzed the overall WOMAC scores in NEM® supplemented patients with knee OA classified according to Kellgren-Lawrence score at baseline and 10, 30, and 60 days post-treatment as shown in Table 3. Patients with KL grade 1 had significantly lower overall WOMAC scores compared to those with KL grade 2 ($p=0.018$) and KL grade 3 ($p=0.001$) at baseline. After 30 days of NEM® supplementation overall WOMAC scores remained significantly higher in patients with KL grade 3 compared to those with KL grade 1 ($p=0.002$) and KL grade 2 ($p=0.001$). Importantly, at 60 days following NEM® supplementation there were no differences in overall WOMAC scores between patients with KL grade 1, grade 2 and grade 3.

Effects of NEM® supplementation on joint pain and overall disease activity

Assessment of joint pain using VAS revealed that supplementation with NEM® resulted in significant reduction of pain from baseline at all-time points. Moreover, pain significantly decreased on VAS between all-time points over the study period as shown in Table 4. In comparison to baseline values, significant reduction of pain was observed at 10 days (19.6% reduction, $p<0.0001$), at 30 days (31.8% reduction, $p<0.0001$) and at 60 days (49.0% reduction, $p<0.0001$) following NEM® supplementation. The similar rate of reduction of VAS

| Days post-treatment | Pain | Stiffness | Function | Overall |
|---------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|
| Baseline | 8.1 ± 0.3 ^{a, b, c} | 3.1 ± 0.2 ^{a, b, c} | 30.4 ± 1.0 ^{a, b, c} | 41.5 ± 1.4 ^{a, b, c} |
| 10 days | 6.5 ± 0.4 ^{a, d, e} | 2.7 ± 0.2 ^{a, d, e} | 25.4 ± 1.1 ^{a, d, e} | 34.6 ± 1.6 ^{a, d, e} |
| 30 days | 5.5 ± 0.4 ^{b, d, f} | 2.0 ± 0.2 ^{b, d, f} | 20.8 ± 1.2 ^{b, d, f} | 28.3 ± 1.6 ^{b, d, f} |
| 60 days | 3.9 ± 0.4 ^{c, e, f} | 1.5 ± 0.2 ^{c, e, f} | 16.7 ± 1.2 ^{c, e, f} | 22.1 ± 1.6 ^{c, e, f} |

Results are presented as mean ± SEM (n=70). $p<0.05$: ^abaseline vs. 10 days; ^bbaseline vs. 30 days; ^cbaseline vs. 60 days; ^d10 vs. 30 days; ^e10 vs. 60 days; ^f30 vs. 60 days; p values were determined by repeated measures univariate analysis of variance (RM-ANOVA, post hoc Bonferroni)

Table 2: WOMAC scores in NEM® supplemented patients with knee OA at baseline and after 10, 30 and 60 days of therapy.

pain and pain related WOMAC scores was noticed from baseline at all-time point as shown in Figure 1.

Patients' evaluation of disease activity using VAS revealed significant treatment response from baseline at all-time points. Decrease of disease activity was observed after 10 days by 17.9% ($p < 0.0001$), after 30 days by 32.7% ($p < 0.0001$) and after 60 days by 46.2% ($p < 0.0001$) of NEM® therapy. A significant decrease of disease activity was observed when comparison was done between all-time points over the study period (Table 4).

Supplementation with NEM® resulted in a significant decrease in disease activity from baseline at all-time points as estimated by physicians. Decrease of disease activity was observed at 10 days by 21.6% ($p < 0.0001$), at 30 days by 40.9% ($p < 0.0001$) and by 53.1% ($p < 0.0001$) at 60 days post-treatment in comparison to baseline values.

Disease activity significantly decreased when comparisons were done between each time points over the study period (Table 4).

There was no significant difference between patient's and physician's assessment of disease activity using VAS.

Effects of NEM® supplementation on Lequesne knee osteoarthritis indices

Supplementation with NEM® resulted in a significant treatment response as reflected by significantly decreased overall Lequesne scores from baseline at all-time points. Moreover, overall Lequesne scores significantly decreased when comparisons were done between all-time points over the study period as shown in Table 5. A significant decrease in overall Lequesne scores was observed after 10 days (11.2% improvement, $p = 0.0002$), 30 days (24.0% improvement, $p < 0.0001$) and

| Days post-treatment | Grade 1 (n=16) | Grade 2 (n=32) | Grade 3 (n=22) |
|---------------------|----------------------------|-------------------------|----------------------------|
| Baseline | 33.2 ± 1.7 ^{a, b} | 42.4 ± 2.2 ^a | 46.3 ± 2.1 ^b |
| 10 days | 31.2 ± 1.6 | 32.2 ± 2.5 | 40.6 ± 3.1 |
| 30 days | 23.3 ± 1.9 ^b | 24.4 ± 2.3 ^c | 37.7 ± 2.8 ^{b, c} |
| 60 days | 18.8 ± 2.2 | 20.1 ± 2.7 | 27.4 ± 2.8 |

Results are presented as mean ± SEM (n=70), $p < 0.05$: ^agrade 1 vs. grade 2; ^bgrade 1 vs. grade 3; ^cgrade 2 vs. grade 3; p values were determined by repeated measures univariate analysis of variance (RM-ANOVA, post hoc Bonferonni)

Table 3: Overall WOMAC scores in NEM® supplemented patients with knee OA classified according to Kellgren-Lawrence score at baseline and at 10, 30 and 60 days after therapy.

| Days post-treatment | VAS pain | VAS disease activity (patients) | VAS disease activity (physicians) |
|---------------------|-------------------------------|---------------------------------|-----------------------------------|
| Baseline | 53.1 ± 1.7 ^{a, b, c} | 49.8 ± 1.9 ^{a, b, c} | 50.1 ± 1.5 ^{a, b, c} |
| 10 days | 42.7 ± 2.2 ^{a, d, e} | 40.9 ± 2.2 ^{a, d, e} | 39.3 ± 2.0 ^{a, d, e} |
| 30 days | 36.2 ± 2.4 ^{b, d, f} | 33.5 ± 2.2 ^{b, d, f} | 29.6 ± 2.0 ^{b, d, f} |
| 60 days | 27.1 ± 2.4 ^{c, e, f} | 26.8 ± 2.5 ^{c, e, f} | 23.5 ± 2.2 ^{c, e, f} |

Results are presented as mean ± SEM (n=70), $p < 0.05$: ^abaseline vs. 10 days; ^bbaseline vs. 30 days; ^cbaseline vs. 60 days; ^d10 vs. 30 days; ^e10 vs. 60 days; ^f30 vs. 60 days; p values were determined by repeated measures univariate analysis of variance (RM-ANOVA, post hoc Bonferonni)

Table 4: Joint pain, patient's and physician's disease activity VAS in NEM® supplemented patients with knee OA at baseline and at 10, 30, and 60 days after therapy.

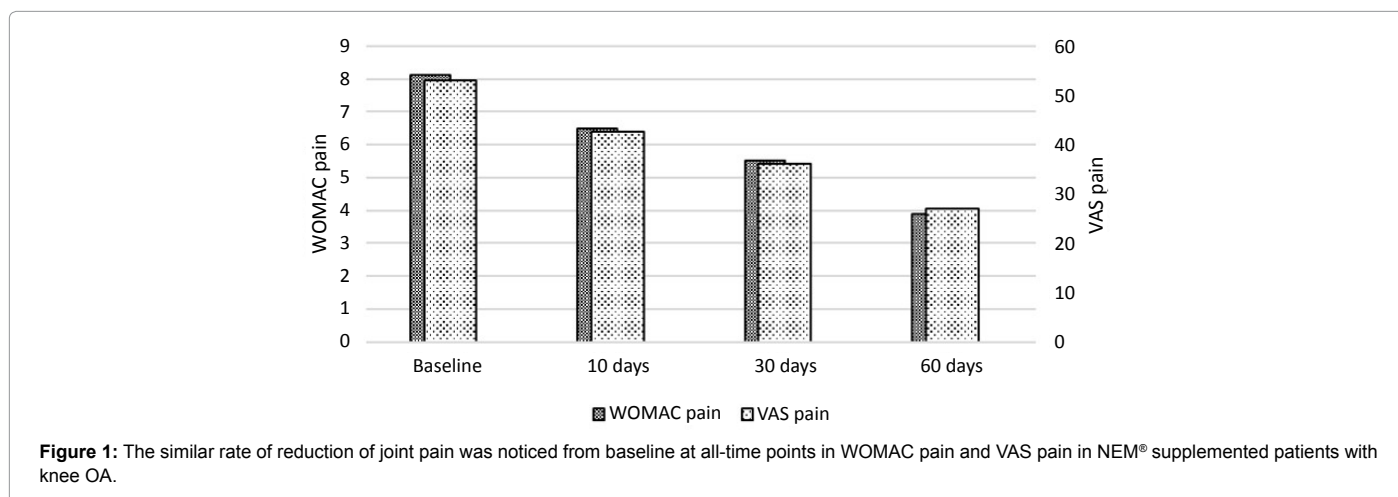


Figure 1: The similar rate of reduction of joint pain was noticed from baseline at all-time points in WOMAC pain and VAS pain in NEM® supplemented patients with knee OA.

| Days post-treatment | Pain or Discomfort | Maximum Distance Walked | Activities of Daily Living | Overall |
|---------------------|------------------------------|---------------------------|------------------------------|-------------------------------|
| Baseline | 5.1 ± 0.2 ^{a, b, c} | 3.2 ± 0.2 ^{b, c} | 4.2 ± 0.1 ^{a, b, c} | 12.5 ± 0.4 ^{a, b, c} |
| 10 days | 4.3 ± 0.2 ^{a, d, e} | 3.0 ± 0.1 ^{d, e} | 3.8 ± 0.2 ^{a, d, e} | 11.1 ± 0.4 ^{a, d, e} |
| 30 days | 3.8 ± 0.2 ^{b, d, f} | 2.7 ± 0.1 ^{b, d} | 3.1 ± 0.2 ^{b, d, f} | 9.5 ± 0.5 ^{b, d, f} |
| 60 days | 3.1 ± 0.2 ^{c, e, f} | 2.3 ± 0.2 ^{c, e} | 2.5 ± 0.1 ^{c, e, f} | 7.9 ± 0.5 ^{c, e, f} |

Results are presented as mean ± SEM (n=70), $p < 0.05$: ^abaseline vs. 10 days; ^bbaseline vs. 30 days; ^cbaseline vs. 60 days; ^d10 vs. 30 days; ^e10 vs. 60 days; ^f30 vs. 60 days; p values were determined by repeated measures univariate analysis of variance (RM-ANOVA, post hoc Bonferonni)

Table 5: Lequesne scores in NEM® supplemented OA patients at baseline and at 10, 30 and 60 after therapy.

60 days (36.8% improvement, $p < 0.0001$) of NEM® therapy compared to baseline overall Lequesne scores.

Analysis of pain or discomfort related Lequesne scores revealed significant reduction of pain from baseline at all-time points. Moreover, pain related Lequesne scores significantly decreased between all-time points over the study period (Table 5). Reduction of pain was observed after 10 days (15.7% reduction, $p = 0.0002$), 30 days (25.5% reduction, $p < 0.0001$) and 60 days (39.2% reduction, $p < 0.0001$) following NEM® supplementation.

Supplementation with NEM® resulted in significantly lower distance related Lequesne scores from baseline at 30 days (15.6% improvement, $p = 0.01$) and at 60 days (28.1% improvement, $p < 0.0001$). The improvement of distance related Lequesne scores was not significant from baseline at 10 days (6.3% improvement, $p = 0.64$) (Table 5).

Post-treatment Lequesne activities of daily living related scores were significantly lower at all-time points compared to baseline values. Namely, mean Lequesne activities of daily living subscores showed a 9.5% reduction at 10 days ($p = 0.002$), 26.2% reduction at 30 days ($p < 0.0001$) and 40.5% reduction at 60 days ($p < 0.0001$).

Next, we analyzed the overall Lequesne scores in NEM® supplemented patients with knee OA classified according to Kellgren-Lawrence score at baseline and after 10, 30, and 60 days of NEM® treatment as shown in Table 6. Patients with KL grade 3 had significantly higher overall Lequesne scores compared to those with KL grade 1 ($p < 0.0001$) and KL grade 2 ($p < 0.0001$) at baseline. This difference remained at 30 days post-treatment as overall Lequesne scores were significantly higher in patients with KL grade 3 compared to those with KL grade 1 ($p < 0.0001$) and KL grade 2 ($p < 0.0001$). Of note, there were no differences in overall Lequesne scores between patients with KL grade 1, grade 2 and grade 3 after 60 days of NEM® supplementation.

Correlation analysis revealed the significant association of patients' age, BMI, and disease duration with overall Lequesne scores at baseline and at all-time points over the study period as shown in Table 7.

Global assessment of NEM® efficacy and analgesic use

In a Global Assessment, greater than 68% of patients rated

| Days post-treatment | Grade 1 (n=16) | Grade 2 (n=32) | Grade 3 (n=22) |
|---------------------|-------------------------|-------------------------|---------------------------|
| Baseline | 10.4 ± 0.8 ^b | 11.6 ± 0.6 ^c | 15.2 ± 0.7 ^{b,c} |
| 10 days | 10.0 ± 0.7 ^b | 9.8 ± 0.6 ^c | 13.9 ± 0.7 ^{b,c} |
| 30 days | 8.1 ± 0.8 ^b | 8.2 ± 0.5 ^c | 12.5 ± 0.8 ^{b,c} |
| 60 days | 7.3 ± 0.8 | 7.1 ± 0.7 | 9.6 ± 0.7 |

Results are presented as mean ± SEM (n=70), $p < 0.05$: ^agrade 1 vs. grade 2; ^bgrade 1 vs. grade 3; ^cgrade 2 vs. grade 3; p values were determined by repeated measures univariate analysis of variance (RM-ANOVA, post hoc Bonferroni)

Table 6: Overall Lequesne scores in NEM® supplemented OA patients classified according to Kellgren-Lawrence score at baseline and at 10, 30 and 60 days after therapy.

| | Lequesne score | | | |
|-------------|----------------|------------|------------|------------|
| | baseline | at 10 days | at 30 days | at 60 days |
| BMI | 0.325** | 0.295* | 0.321** | 0.344** |
| OA duration | 0.298* | 0.245* | 0.261* | N.S. |
| Age | N.S. | 0.349** | 0.361** | 0.313** |

Pearson correlation coefficient; * $p < 0.05$; ** $p < 0.01$, N.S.; non-significant

Table 7: Correlations between patients' age, BMI and disease duration and overall Lequesne scores at baseline and after 10, 30, and 60 days of NEM® supplementation.

the efficacy of NEM® as good or excellent following 60 days of supplementation (Table 8). Physicians also rated the treatment effective in subjects, and greater than 78% of physicians rated the efficacy of NEM® as good or excellent (Table 9).

More than 98% of patients rated the safety and tolerability of NEM® as good or excellent (Table 8) and all physicians rated the safety and tolerability of NEM® as good or excellent (Table 9). Prior to study commencement, patients consumed on average 1.50 ± 0.11 (mean ± SEM) tablets of paracetamol per day. Analgesic use had dropped considerably to 0.44 ± 0.08 ($p < 0.001$) tablets per day at 60 days of supplementation with NEM®. The use of paracetamol was not significantly reduced after 10 days, but the significant reduction was observed after 30 days (55.3%; $p < 0.001$) and 60 days (70.7%; $p < 0.001$) following the treatment with NEM®.

There were no serious adverse events reported during the study. There were three adverse events reported, one was a skin rash which was not related to allergic reaction to medication, constipation and stomach discomfort. All three reported adverse events were mild and transient and they might not be related to the study material. All three patients completed the 2-month study per the protocol.

Discussion

Osteoarthritis is a common disease and it is estimated that about one-third of the population have some form of OA in European countries [27,28]. Patients with OA experience varying degrees of chronic pain and joint stiffness which largely contributes to functional impairment and decreased quality of life [29]. It is expected that the incidence of OA will increase worldwide as population ages. Therefore, it is important that OA patients have effective and safe treatment options.

This clinical trial was designed to evaluate the efficacy and safety of NEM® as a treatment option for knee OA. Results from this study suggest that NEM®, 500 mg taken once daily, is both effective and safe for management pain associated with knee OA and considerably improves flexibility of the affected joints. NEM® has the added benefit as the use of analgesics significantly dropped over the study period. This study demonstrated that NEM® supplementation resulted in rapid

| Patient's Global Assessment | | | | |
|-----------------------------|----------|-----------|-------------------------|-----------|
| | Efficacy | | Safety and Tolerability | |
| | Number | Frequency | Number | Frequency |
| Excellent | 30 | 42.90% | 62 | 88.60% |
| Good | 18 | 25.70% | 7 | 10.00% |
| Moderate | 16 | 22.80% | 1 | 1.40% |
| Bad | 3 | 4.30% | 0 | 0.00% |
| No effect | 3 | 4.30% | 0 | 0.00% |

Table 8: Patient's Global Assessment of efficacy and safety and tolerability following 60 days of NEM® supplementation.

| Physician's Global Assessment | | | | |
|-------------------------------|----------|-----------|-------------------------|-----------|
| | Efficacy | | Safety and Tolerability | |
| | Number | Frequency | Number | Frequency |
| Excellent | 30 | 42.90% | 66 | 94.30% |
| Good | 25 | 35.70% | 4 | 5.70% |
| Moderate | 13 | 18.50% | 0 | 0.00% |
| Bad | 2 | 2.90% | 0 | 0.00% |
| No effect | 0 | 0.00% | 0 | 0.00% |

Table 9: Physician's Global Assessment of efficacy and safety and tolerability following 60 days of NEM® supplementation

(10 days) responses for WOMAC pain (19.8% reduction) and stiffness (12.9% reduction). The reduction of pain and stiffness was continuous over the study period and by the end of the follow-up period (60 days) the reduction of pain and stiffness was substantial (51.9% reduction and 51.6% reduction, respectively). By the end of the follow-up period (60 days) the mean response for function had markedly improved (45.1%). The primary outcome measures as assessed by overall WOMAC and Lequesne indices showed significant treatment effects (46.7% improvement and 36.8% improvement, respectively). Evaluation of VAS pain also revealed rapid (10 days) responses (19.6% reduction) and further reduction at 60 days (49.0%). Patient's and physician's evaluation of disease activity utilizing VAS was similar and showed significant treatment response by the end of the follow-up period (46.2% improvement and 53.1% improvement, respectively). In a Global Assessment, the majority of patients (68.6%) and physicians (78.6%) rated the efficacy of NEM® as excellent or good. According to published criteria for a response to treatment for osteoarthritis [30] treatment response to NEM® may be classified as an improvement in WOMAC pain and function as an absolute increase in the mean response rate of 35% after supplementation is considered a clinically meaningful treatment effect [10].

Our findings are in accordance to results from previous clinical studies of NEM® supplementation in patients with OA that were conducted in USA, Germany and Italy [9-12]. The treatment response rates in these studies ranged from 33% to 73% improvement of pain. In agreement with our results, in these studies the statistically significant treatment effects was observed after only 10 days following NEM® supplementation. The variations in treatment rates reported in these studies may be related to differences in OA severity and mean pain and function scores at baseline.

In line with previous studies, our findings confirm excellent safety profile for NEM® as there were no reports of adverse events or serious adverse events associated with treatment. There are no known side effects of NEM®, except from obvious egg allergy concern. This is of key importance as OA is a condition that requires long-term treatment. The common treatment option for OA is analgesics and NSAIDs which are known to lead to gastric [31] and cardiovascular [32] complications. The reduction of use of these drugs adds to the safety benefit of NEM® supplementation.

In this clinical trial no subjects withdrew from the study and there was good treatment compliance. This was open-label clinical study therefore the placebo effect cannot be excluded. We believe that this limitation is minor when considering the convincing clinical evidence for the beneficial effects of NEM® in the treatment of knee osteoarthritis.

Conclusion

The results from this open-label clinical study demonstrate that NEM® may be a viable therapeutic option for the management of pain and stiffness associated with osteoarthritis of the knee. In this clinical study, NEM®, 500 mg taken once daily, significantly reduced both pain and stiffness rapidly (10 days) and this effect continued to improve through 60 days of NEM® supplementation. This beneficial effect of NEM® was accompanied with significant reduction in the amount of analgesic consumed during the study period. NEM® supplementation has shown to be both safe and effective in managing knee osteoarthritis.

Highlights

- NEM® supplementation significantly reduced pain, stiffness and functional disability in patients with moderate and severe knee OA

- NEM® supplementation was safe and well tolerated
- NEM® supplementation was accompanied with the reduction of the amount of analgesic consumed by patients with knee OA

Acknowledgment

The study sponsor was Pharmanova, Belgrade, Serbia.

Conflict of Interest

ND, SN, MB, AKN, KV, NP received consulting fees from Pharmanova, Serbia. VK is employed by the study sponsor (Pharmanova, Serbia). The authors report no other conflicts of interest in this work.

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