

# NEM<sup>®</sup> Brand Eggshell Membrane Effective in the Treatment of Pain and Stiffness Associated with Osteoarthritis of the Knee in an Italian Study Population

Emy Brunello<sup>1</sup>, Antonio Masini<sup>2\*</sup>

<sup>1</sup>Health Department, "REGENESIS" Medical Center, Bovolone, Italy

<sup>2</sup>Complementary Medicine R & D, Phytonaturesas, Bovolone, Italy

Email: [drmasini@phytonature.it](mailto:drmasini@phytonature.it), [emy.brunello@teletu.it](mailto:emy.brunello@teletu.it)

Received 19 January 2016; accepted 24 February 2016; published 29 February 2016

Copyright © 2016 by authors and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

---

## Abstract

A single-center, open-label clinical study was conducted to evaluate the efficacy and safety of NEM<sup>®</sup> as a natural treatment for pain and stiffness associated with osteoarthritis of the knee in an Italian population. NEM<sup>®</sup> brand eggshell membrane is a unique dietary supplement that contains naturally occurring glycosaminoglycans and proteins essential for maintaining healthy joints and connective tissues. Twenty-five subjects received oral NEM<sup>®</sup>, 500 mg once daily for four weeks. The primary outcome measure was to evaluate the mean effectiveness of NEM<sup>®</sup> in relieving general pain associated with moderate osteoarthritis of the knee at 10 and 30 days utilizing a 10-question, abbreviated questionnaire based on the WOMAC osteoarthritis questionnaire. Supplementation with NEM<sup>®</sup> produced a significant treatment response from baseline at both 10 days and 30 days for composite pain (40.6% reduction,  $p < 0.001$ ; 66.4% reduction,  $p < 0.001$ , respectively). There was also a statistically significant concurrent reduction in analgesic use during the 30-day study period. Additionally, a significant treatment response from baseline was also observed for composite stiffness at both 10 days and 30 days (22.2% reduction,  $p = 0.009$ ; 59.7% reduction,  $p < 0.001$ , respectively). There were no adverse events or serious adverse events reported during the study and the treatment was reported to be well tolerated by study participants. NEM<sup>®</sup> is an effective and safe natural therapeutic option for the treatment of both pain and stiffness associated with osteoarthritis of the knee. Supplementation with NEM<sup>®</sup>, 500 mg taken once daily, significantly reduced both pain and stiffness rapidly (10 days) and this effect continued to improve through 30 days. There was also a meaningful reduction in the amount of analgesic consumed on a weekly basis, which further enhanced patients' safety.

\*Corresponding author.

## Keywords

**Knee, Osteoarthritis, Supplement, Egg Shell Membrane, Glycosaminoglycans**

### 1. Introduction

Osteoarthritis (OA) is a degenerative disease primarily affecting the cartilage of articular joints and is frequently accompanied by varying degrees of joint pain and stiffness in afflicted subjects. OA is one of the most common causes of chronic pain in adults 65 and older and often leads to disability as the disease progresses [1]. Estimates of the prevalence of OA in European populations vary widely, however, two recent studies conducted in Italy found the prevalence of knee OA to be 29.8% [2] and 36.7% [3]. The pain associated with these maladies can be quite debilitating and few treatment options exist outside of easing symptoms. This usually involves the use of analgesics (e.g. acetaminophen, hydrocodone) or non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. ibuprofen, celecoxib, etc.), alone or in combination. Most of these treatments have shown limited effectiveness in randomized controlled clinical trials (RCTs) [4]-[6] or are known to have significant and sometimes severe side effects [7] [8]. NEM<sup>®</sup> brand eggshell membrane has previously demonstrated good efficacy in relieving joint pain and stiffness in multiple clinical trials in the U.S. [9] [10] and recently in a German population [11].

Eggshell membrane is primarily composed of fibrous proteins such as Collagen Type I [12]. However, eggshell membranes have also been shown to contain other bioactive components, namely glycosaminoglycans (*i.e.* dermatan sulfate [13], chondroitin sulfate [13], hyaluronic acid [14], etc). ESM Technologies, LLC (Carthage, MO, USA) has developed methods to efficiently and effectively separate eggshell membrane from eggshells on a commercial metric-ton scale. The isolated membrane is then partially hydrolyzed using a proprietary process and dry-blended to produce NEM<sup>®</sup> brand eggshell membrane. Compositional analysis of NEM<sup>®</sup> conducted by ESM Technologies has identified a high content of protein and moderate quantities of glucosamine (up to 1% by dry weight), chondroitin sulfate (up to 1%), hyaluronic acid (up to 2%), and collagen (Type I, up to 5%).

The single-center trial reported here was designed to evaluate the efficacy of this natural arthritis treatment in an Italian population and to confirm the results found previously in the U.S. and Germany. Therefore, a 1-month open-label study was conducted at a single clinical site in Italy to evaluate the efficacy and tolerability of NEM<sup>®</sup> for the relief of the pain and discomfort associated with osteoarthritis of the knee.

### 2. Patients and Methods

#### 2.1. Study Design

The study was conducted according to a prospective, single-center, open-label design and was conducted in Italy in accordance with the International Conference on Harmonization guideline for the principles of Good Clinical Practice (ICH E6) and the Declaration of Helsinki. Patients provided their written informed consent to participate. The clinical investigators were not blinded to treatment (open-label). Treatment consisted once daily orally of 500 mg of NEM<sup>®</sup> in vegetarian capsules that were stored in closed containers at ambient temperature. Clinic visits were scheduled for subjects at study initiation and at 10 days and 30 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 medications. Analgesics (*i.e.* acetaminophen) were allowed for pain relief, as needed. Subjects recorded the time and amount of analgesic taken in patient diaries.

#### 2.2. Patients

All subjects 18 years of age or older who were seeking relief of mild to moderate pain due to osteoarthritis of the knee were considered for enrollment in the study. In order to be eligible, subjects must have had moderate persistent pain in the knee associated with osteoarthritis and must have had baseline scores within the range of 4 - 7 on questions 1, 2, & 5 dealing with joint pain. Subjects that were currently taking analgesic medications daily, currently taking glucosamine, chondroitin sulfate, MSM, or collagen were ineligible to participate in the study. Patients were excluded if they were currently receiving remission-inducing drugs such as methotrexate or im-

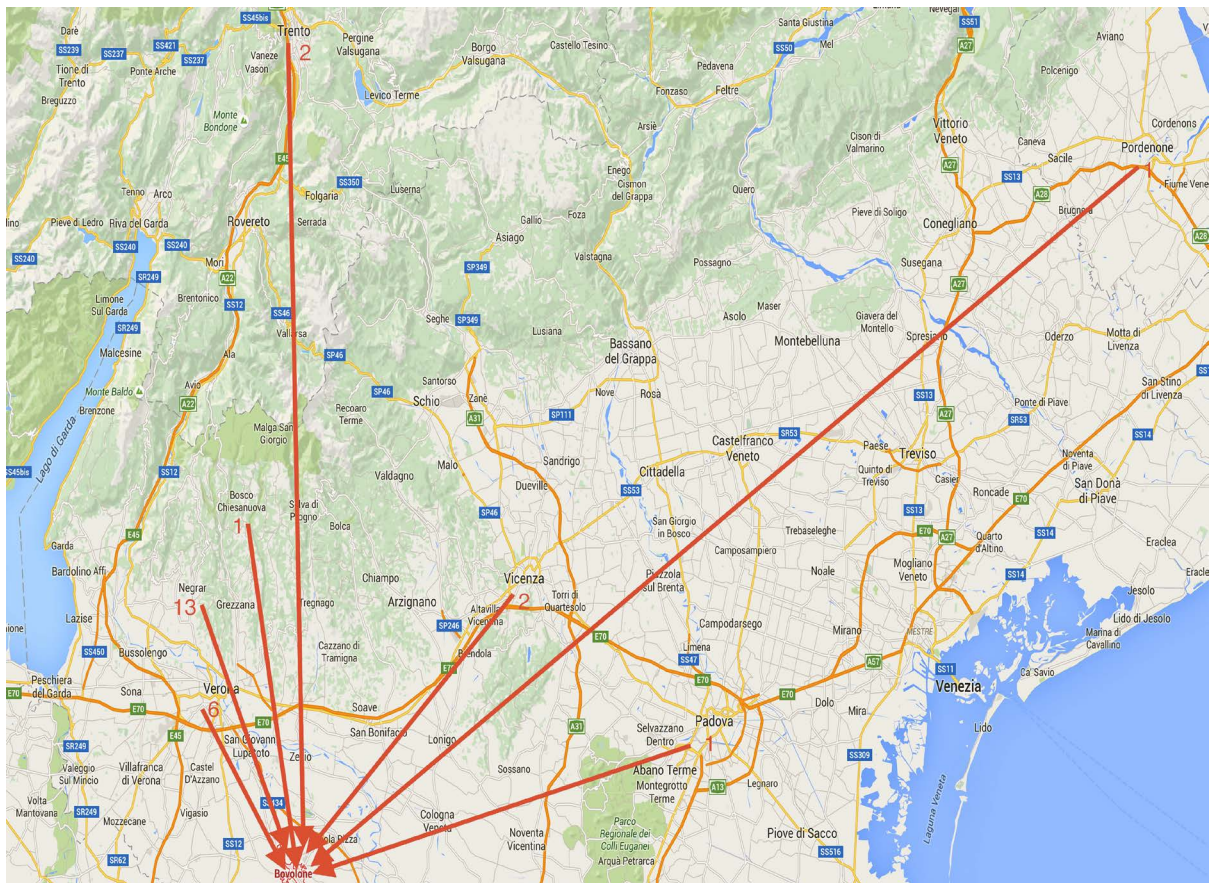
munosuppressive medications or had received them within the past 3 months. Other exclusionary criteria were: a known allergy to eggs or egg products, or pregnant or breastfeeding women. Subjects participating in any other research study involving an investigational product (drug, device, or biologic) or a new application of an approved product, within 30 days of screening were also excluded from participating in the trial.

### 2.3. Location of Patients

The majority of patients were enrolled in an area of 50 km around the city of Verona, the capital of the homonymous province, where the Medical center REGENESIS is located, others patients come from different provinces, in particular Trento, Vicenza, Padova and Pordenone. The map below shows the different origins of the patients.

### 2.4. Treatment Response

The primary outcome measure of this study was to evaluate the mean effectiveness of NEM<sup>®</sup> in relieving general pain associated with moderate osteoarthritis of the knee (composite score of Questions 1 - 8). A composite score was calculated as the sum of the questions of interest. Additional outcome measures were to evaluate general stiffness (composite score of Questions 9 & 10), analgesic use during the study, and non-composite mean results for all 10 individual questions. The primary treatment response endpoints were the 10-and 30-day patient assessments utilizing a 10-question short-form questionnaire (see **Figure 1**) derived from the Western Ontario & McMasters Universities Osteoarthritis Index (WOMAC) questionnaire. Each question included a zero to 10 analog Likert-scale, with zero equating to no pain (or no stiffness) and 10 equating to most severe pain (or most severe stiffness). Patients were asked to mark a number corresponding to the perceived pain (or stiffness) from the affected treatment joint (s). Endpoints were then compared to pretreatment assessments.



**Figure 1.** Questions used in the short-form questionnaire completed by study participants.

- 
- Question 1: *Pain when walking on level ground?*
- Question 2: *Pain when going up or down stairs?*
- Question 3: *Pain while at rest (i.e. sitting, lying down, etc.)?*
- Question 4: *Pain when sitting with legs bent for an extended period of time (i.e. in a car, at a theater, etc.)?*
- Question 5: *Pain when getting up from a seated position?*
- Question 6: *Pain when getting in and out of a car, a bathtub, etc.?*
- Question 7: *Pain when bending, stooping, or kneeling?*
- Question 8: *Pain when putting on socks or pantyhose?*
- Question 9: *Stiffness when first getting up from bed in the morning?*
- Question 10: *Stiffness when sitting, laying, or resting later in the day?*
- 

## 2.5. Adverse Events

A secondary objective of this study was to evaluate tolerability and any adverse reactions associated with supplementation with NEM<sup>®</sup>. The subjects' self-assessment diaries were reviewed and any discomfort or other adverse events were recorded and reported in accordance with applicable ICH Guidelines. Adverse events and serious adverse events were assessed by the clinical investigator at each study visit and followed until resolution, as necessary. Serious adverse events were required to be reported to the clinical investigator immediately.

## 2.6. Statistical Analysis

As this was an open-label study, a simple single-group sample size estimate [15] was performed for statistical power determination for a continuous variable. In the similar trial with NEM<sup>®</sup> conducted in Germany [11], the mean standard deviation for the study subjects for pain was 1.55 points. We hoped to be able to detect a 1.5 point difference from baseline within the 10-point Likert scale. Therefore a minimum of 18 subjects would need to be enrolled to have a 95% likelihood of detecting the expected improvement with a statistical power of 80%. Post-baseline statistical analyses were done as repeated measures Analysis of Variance (rm-ANOVA). Items found to have statistical significance with rm-ANOVA were then compared using a Wilcoxon test for dependent samples. Statistical significance was accepted at  $p < 0.05$ . Analysis of the primary outcome measure (the change from baseline in general pain levels) was conducted in the per protocol population. SPSS Statistics V19.0 was used for all statistical analyses [16].

## 3. Results

Patient recruitment began in May 2014 at a single clinical site in Italy and the final follow-up was conducted in July 2014. A total of twenty-five subjects between the ages of 43 and 81 were enrolled with osteoarthritis of the knee. Of these subjects, twenty (80%) were female and five (20%) were male. Of the twenty-five subjects with knee OA, 10 (40.0%) had bilateral incidence. Patient demographics are reported in **Table 1**. All twenty-five

**Table 1.** Patient demographics.<sup>a</sup>

|                        |                |
|------------------------|----------------|
| Age, yrs               | 69.4 ± 9.6     |
| Sex                    |                |
| Male (%)               | 5 (20)         |
| Female (%)             | 20 (80)        |
| Height, cm             | 165.8 ± 7.0    |
| Weight, kg             | 71.0 ± 10.5    |
| Body-mass Index        | 25.8 ± 3.2     |
| Affected Joint         |                |
| Knee (l, r, bilateral) | 25 (5, 10, 10) |

a. Except where indicated otherwise, values are reported as mean ± standard deviation (SD) (n = 25). BMI was determined as weight in kilograms divided by height in meters squared.

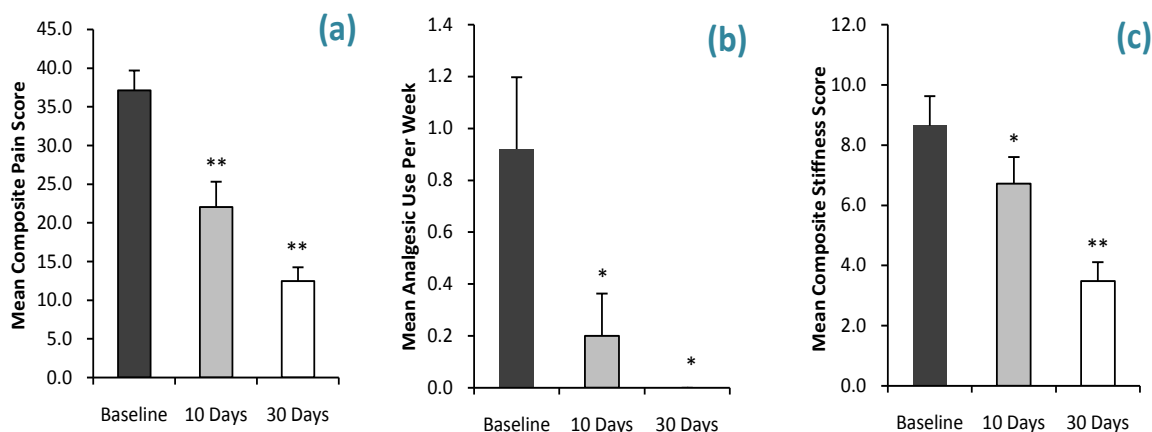
subjects completed the one month study per the protocol. Compliance with the study treatment regimen was good.

A clinical comparison of valid subjects was carried out to obtain mean baseline scores for each of the ten questions from the subject questionnaire, as well as the 10-day and 30-day endpoints. Statistical analysis of the primary outcome measure revealed that supplementation with NEM<sup>®</sup> produced a significant treatment response from baseline at both 10 days and 30 days for composite pain (40.6% reduction,  $p < 0.001$ ; 66.4% reduction,  $p < 0.001$ , respectively) (see **Figure 2(a)**). There was also a statistically significant concurrent reduction in analgesic use during the 30-day study period. At baseline, subjects consumed analgesic slightly less than one day per week on average, and this dropped 78.3% ( $p = 0.017$ ) to 0.2 days through the 10-day endpoint. All 25 subjects consumed no analgesic through the final 3 weeks of the study ( $p = 0.003$ ) (see **Figure 2(b)**). A significant treatment response from baseline was also observed for composite stiffness at both 10 days and 30 days (22.2% reduction,  $p = 0.009$ ; 59.7% reduction,  $p < 0.001$ , respectively) (see **Figure 2(c)**). Supplementation with NEM<sup>®</sup> also produced a significant treatment response from baseline after 10 days when replying to Questions 1 - 5 & 7 - 8 (30.2% to 50.0% improvement) and at 30 days for all eight pain-related questions evaluated (50.9% to 78.9% improvement) (see **Table 2**). Treatment response fell shy of statistical significance for Question 6 at 10 days ( $p = 0.190$ ). Similarly, a significant treatment response for stiffness was found at 10 days (Question 9) (27.7% improvement) but fell just shy of significance for Question 10 (15.4% improvement,  $p = 0.069$ ). There was also a significant treatment response at 30 days for both stiffness-related questions (Q9 & Q10) (53.2% & 69.2%, respectively). There were no adverse events or serious adverse events reported during the study and the treatment was reported to be well tolerated by study participants.

#### 4. Discussion

Osteoarthritis is very common in Italy with about one-third of the population having some form of the disease [2] [3]. This has a large impact on the quality of life of those afflicted with OA [17]. This open-label clinical trial was designed to evaluate the efficacy of NEM<sup>®</sup> as a natural arthritis treatment in an Italian population and to further validate the extension of the body of clinical evidence for NEM<sup>®</sup> from the United States to the general European population. The study demonstrated that NEM<sup>®</sup> is effective and safe for treating both pain and stiffness associated with osteoarthritis of the knee and results in the use of less analgesic medication.

Study subjects experienced relatively rapid (10 days) responses for both composite pain (40.6% improvement) and composite stiffness (22.2% improvement). By the end of the follow-up period (30 days) the mean response for composite pain and stiffness had increased substantially (66.4% improvement & 59.7% improvement, respectively). These results are quite similar to results from previous clinical studies of NEM<sup>®</sup> that were conducted in the U.S. [9] [10] and is a somewhat larger effect than what was found recently in a German population [11]. This difference may be a result of a small difference in mean pain at baseline between the two study populations (4.6 compared to 4.9 in Germany). Both studies showed statistically significant treatment effects at 10



**Figure 2.** Mean composite pain score (a), mean analgesic user per week (b), and mean composite stiffness score (c) at baseline and 10 & 30 days of supplementation. Values are reported as means  $\pm$  standard deviation (SD) ( $n = 25$ ). \* $p > 0.05$ , \*\* $p < 0.001$

**Table 2.** Mean values by question in an NEM-supplemented treatment group at baseline and 10 & 30 days post-treatment.

|            | Days Post-Treatment | Mean $\pm$ SD | Percent Improvement | P-value <sup>a</sup> |             | Days Post-Treatment | Mean $\pm$ SD | Percent Improvement | P-value <sup>a</sup> |
|------------|---------------------|---------------|---------------------|----------------------|-------------|---------------------|---------------|---------------------|----------------------|
| Question 1 | Baseline (n = 25)   | 5.1 $\pm$ 0.3 | -                   | -                    | Question 6  | Baseline (n = 25)   | 2.9 $\pm$ 0.6 | -                   | -                    |
|            | 10 (n = 25)         | 3.1 $\pm$ 0.5 | 39.2%               | <0.001**             |             | 10 (n = 25)         | 2.2 $\pm$ 0.6 | 24.1%               | 0.190                |
|            | 30 (n = 25)         | 2.0 $\pm$ 0.5 | 60.8%               | <0.001**             |             | 30 (n = 25)         | 0.9 $\pm$ 0.2 | 69.0%               | 0.002*               |
| Question 2 | Baseline (n = 25)   | 5.6 $\pm$ 0.4 | -                   | -                    | Question 7  | Baseline (n = 25)   | 5.3 $\pm$ 0.6 | -                   | -                    |
|            | 10 (n = 25)         | 3.1 $\pm$ 0.6 | 44.6%               | <0.001**             |             | 10 (n = 25)         | 3.7 $\pm$ 0.5 | 30.2%               | 0.005*               |
|            | 30 (n = 25)         | 1.7 $\pm$ 0.4 | 69.6%               | <0.001**             |             | 30 (n = 25)         | 2.6 $\pm$ 0.3 | 50.9%               | <0.001**             |
| Question 3 | Baseline (n = 25)   | 3.2 $\pm$ 0.6 | -                   | -                    | Question 8  | Baseline (n = 25)   | 3.8 $\pm$ 0.6 | -                   | -                    |
|            | 10 (n = 25)         | 1.6 $\pm$ 0.5 | 50.0%               | 0.031**              |             | 10 (n = 25)         | 1.9 $\pm$ 0.5 | 50.0%               | 0.006*               |
|            | 30 (n = 25)         | 0.8 $\pm$ 0.3 | 75.0%               | <0.001**             |             | 30 (n = 25)         | 0.8 $\pm$ 0.3 | 78.9%               | <0.001**             |
| Question 4 | Baseline (n = 25)   | 5.6 $\pm$ 0.4 | -                   | -                    | Question 9  | Baseline (n = 25)   | 4.7 $\pm$ 0.6 | -                   | -                    |
|            | 10 (n = 25)         | 3.5 $\pm$ 0.4 | 37.5%               | <0.001**             |             | 10 (n = 25)         | 3.4 $\pm$ 0.5 | 27.7%               | 0.018*               |
|            | 30 (n = 25)         | 1.9 $\pm$ 0.3 | 66.1%               | <0.001**             |             | 30 (n = 25)         | 2.2 $\pm$ 0.4 | 53.2%               | <0.001**             |
| Question 5 | Baseline (n = 25)   | 5.6 $\pm$ 0.3 | -                   | -                    | Question 10 | Baseline (n = 25)   | 3.9 $\pm$ 0.5 | -                   | -                    |
|            | 10 (n = 25)         | 3.0 $\pm$ 0.5 | 46.4%               | <0.001**             |             | 10 (n = 25)         | 3.3 $\pm$ 0.5 | 15.4%               | 0.069                |
|            | 30 (n = 25)         | 1.8 $\pm$ 0.4 | 67.9%               | <0.001**             |             | 30 (n = 25)         | 1.2 $\pm$ 0.3 | 69.2%               | <0.001**             |

a. P-values were determined by Wilcoxon test for dependent samples following a statistically significant difference as determined by rm-ANOVA, and represent treatment versus baseline. \* $p < 0.05$ , \*\* $p < 0.001$ .

days, so this is not too concerning. Study subjects also experienced large improvements in particular aspects of pain when reviewing the individual questions from the short-form questionnaire. For example, at 30 days there was a 75% improvement in pain while at rest (Question 3) and a 79% improvement in pain when putting on socks or pantyhose (Question 8). Likewise, pain when going up and down stairs (Question 2) and pain when getting in and out of a car, bathtub, etc. (Question 6) were both improved by nearly 70%. This broad treatment effect relating to numerous activities of daily living should have a profound impact on the subjects overall quality of life. This should also help them to remain active as they age, which is also important to other aspects of health (*i.e.* cardiovascular disease, neurodegenerative disease, etc.).

The safety profile for NEM<sup>®</sup> was again found to be excellent as there were no reports of adverse events or serious adverse events associated with treatment. This was comparable to the clinical trials conducted with NEM<sup>®</sup> previously [9]-[11]. No side effects from consuming NEM<sup>®</sup> have so far been identified, excluding the obvious egg allergy concern. This is very important in a disease like osteoarthritis that requires long-term treatment. The analgesics and NSAIDs normally used to treat such conditions are known to lead to gastric [7] and cardiovascular [8] complications which can considerably increase mortality in an elderly population.

The trial had a limited enrollment (25 subjects), however no subjects withdrew from the study and there was good treatment compliance. As the trial was also open-label, there is the obvious issue of the placebo effect. The inclusion of a placebo control would have provided greater clinical clarity, however it would have required a substantially larger study population. These limitations are minor when considering the totality of the available clinical evidence for the use of NEM<sup>®</sup> in joint and connective tissue disorders.

## 5. Conclusion

It is important for patients to have treatment options that are both safe and effective in managing chronic diseases such as osteoarthritis, especially in Italy where about one-third of the population is affected. The reporting of the results from this single-center, open-label clinical study demonstrates that NEM<sup>®</sup> brand eggshell membrane is a viable natural treatment option for the management of osteoarthritis of the knee. In this clinical study, NEM<sup>®</sup>, 500 mg taken once daily, significantly reduced both composite pain and stiffness rapidly (10 days) and this effect continued to improve through 30 days. There was also a meaningful reduction in the amount of analgesic consumed on a weekly basis, which further enhanced patients' safety.

## Acknowledgements

The study was sponsored by Phytonaturesas. Dr. Emy Brunello and Dr. Antonio Masini are employed by the sponsor. The authors would like to acknowledge ESM Technologies, LLC for providing the NEM<sup>®</sup> study material for this trial.

## References

- [1] Peat, G., McCarney, R. and Croft, P. (2001) Knee Pain and Osteoarthritis in Older Adults: A Review of Community Burden and Current Use of Primary Health Care. *Annals of the Rheumatic Diseases*, **60**, 91-97. <http://dx.doi.org/10.1136/ard.60.2.91>
- [2] Mannoni, A., Briganti, M.P., Di Bari, M., Ferrucci, L., Costanzo, S., Serni, U., *et al.* (2003) Epidemiological Profile of Symptomatic Osteoarthritis in Older Adults: A Population Based Study in Dicomano, Italy. *Annals of the Rheumatic Diseases*, **62**, 576-578. <http://dx.doi.org/10.1136/ard.62.6.576>
- [3] Schaap, L.A., Peeters, G.M.E.E., Dennison, E.M., Zambon, S., Nikolaus, T., Sanchez-Martinez, M., *et al.* (2011) European Project on Osteoarthritis (EPOSA): Methodological Challenges in Harmonization of Existing Data From Five European Population-based Cohorts on Aging. *BMC Musculoskeletal Disorders*, **12**, 272. <http://dx.doi.org/10.1186/1471-2474-12-272>
- [4] Altman, R. (1999) Ibuprofen, Acetaminophen and Placebo in Osteoarthritis of the Knee: A Six-Day Double-Blind Study [Abstract]. *Arthritis & Rheumatology*, **42**, S403.
- [5] Case, J.P., Baliunas, A.J. and Block, J.A. (2003) Lack of Efficacy of Acetaminophen in Treating Symptomatic Knee Osteoarthritis. *Archives of Internal Medicine*, **163**, 169-178. <http://dx.doi.org/10.1001/archinte.163.2.169>
- [6] Geba, G.P., Weaver, A.L., Polis, A.B., Dixon, M.E. and Schnitzer, T.J. (2002) Efficacy of Rofecoxib, Celecoxib, and Acetaminophen in Osteoarthritis of the Knee. *Journal of the American Medical Association*, **287**, 64-71. <http://dx.doi.org/10.1001/jama.287.1.64>
- [7] Laine, L. (1996) Nonsteroidal Anti-inflammatory Drug Gastropathy. *Gastrointestinal Endoscopy Clinics of North America*, **6**, 489-504.
- [8] Singh, G., Wu, O., Langhorne, P. and Madhok, R. (2006) Risk of Acute Myocardial Infarction with Nonselective Non-Steroidal Anti-Inflammatory Drugs: A Meta-Analysis. *Arthritis Research & Therapy*, **8**, R153-R162. <http://dx.doi.org/10.1186/ar2047>
- [9] Ruff, K.J., DeVore, D.P., Leu, M.D. and Robinson, M.A. (2009) Eggshell Membrane: A Possible New Natural Therapeutic for Joint and Connective Tissue Disorders. Results from Two Open-Label Human Clinical Studies. *Clinical Interventions in Aging*, **4**, 235-240. <http://dx.doi.org/10.2147/CIA.S5797>
- [10] Ruff, K.J., Winkler, A., Jackson, R.W., DeVore, D.P. and Ritz, B.W. (2009) Eggshell Membrane in the Treatment of Pain and Stiffness from Osteoarthritis of the Knee: A Randomized, Multicenter, Double-Blind, Placebo-Controlled Clinical Study. *Clinical Rheumatology*, **28**, 907-914. <http://dx.doi.org/10.1007/s10067-009-1173-4>
- [11] Danesch, U., Seybold, M., Rittinghausen, R., Treibel, W. and Bitterlich, N. (2014) NEM<sup>®</sup> Brand Eggshell Membrane Effective in the Treatment of Pain Associated with Knee and Hip Osteoarthritis: Results from a Six-Center, Open-Label German Clinical Study. *Journal of Arthritis*, **3**, 136. <http://dx.doi.org/10.4172/2167-7921.1000136>
- [12] Wong, M., Hendrix, M.J.C., von der Mark, K., Little, C. and Stern, R. (1984) Collagen in the Egg Shell Membranes of the Hen. *Developmental Biology*, **104**, 28-36. [http://dx.doi.org/10.1016/0012-1606\(84\)90033-2](http://dx.doi.org/10.1016/0012-1606(84)90033-2)
- [13] Baker, J.R. and Balch, D.A. (1962) A Study of the Organic Material of Hen's-Egg Shell. *Biochemical Journal*, **82**, 352-361. <http://dx.doi.org/10.1042/bj0820352>
- [14] Long, F.D., Adams, R.G. and DeVore, D.P. (2005) Preparation of Hyaluronic Acid from Eggshell Membrane. US Patent No. 6946551.
- [15] National Research Council (2003) Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. National Academies Press, Washington DC. [https://grants.nih.gov/grants/olaw/National\\_Academies\\_Guidelines\\_for\\_Use\\_and\\_Care.pdf](https://grants.nih.gov/grants/olaw/National_Academies_Guidelines_for_Use_and_Care.pdf)
- [16] IBM Corporation, SPSS Statistics. [www.ibm.com](http://www.ibm.com)
- [17] Cook, C., Pietrobon, R. and Hegedus, E. (2007) Osteoarthritis and the Impact on Quality of Life Health Indicators. *Rheumatology International*, **27**, 315-321. <http://dx.doi.org/10.1007/s00296-006-0269-2>