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Mineral rich algae with pine bark improved pain, physical function and analgesic use in mild-knee joint osteoarthritis, compared to Glucosamine: a randomized controlled pilot trial

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Highlights

- Nutraceuticals can be effective for improving osteoarthritis symptoms
- Aq[+] was superior to Glu for improving pain and KOA symptoms
- In females Aq[+] improved functional performance, Glu did not and was correlated with improvements in pain (r=-0.49; R^2=0.24)
- Aq[+] reduced *ad libitum* analgesic use by 72%, compared to Glu

Abstract

Introduction, Osteoarthritis (OA) is characterised by synovial joint pain, functional disability and affects ~13% of people worldwide, of which ~16-27% report Knee-OA (KOA). Glucosamine (Glu) is the most widely used nutraceutical treatment for OA despite a lack of scientific consensus, therefore alternative nutraceutical treatments are required. The aim of this study was to investigate the effect of *Lithothamnion species*, seawater-derived magnesium and pine bark (Aq[+]) on pain, symptoms and improve physical function in symptomatic (sKOA), compared to Glu.

Methods, 358 participants were screened. In a double-blinded crossover pilot-trial, sKOA participant (n=30) were randomly assigned to either the Glu group (2000mg·day^{-1}) or Aq$^+$ (3056mg·day^{-1}) for 12 weeks (clinicaltrials.gov:NCT03106584). The Knee Injury and Osteoarthritis Outcome Score was used to assess subjective pain and symptoms. Timed-up-and-Go (TuG) and Six minute walking distance were used to assess functional change and analgesic use was recorded.

Results, Aq$^+$ improved pain, with a large effect (P<0.01, d'=0.73, 95%CI 0.201-1.265) and no change for Glu (d'=0.38, P=0.06). Only Aq$^+$ improved pain (P<0.05) for males (d'=0.91, 95%CI 0.162-1.667) and females (d'=0.55, 95%CI 0.210-1.299). In females, Aq$^+$ improved TuG by -7.02% (d'=0.92, 95%CI 1.699-0.141) while Glu worsened performance by 4.18% (P=0.04). Aq$^+$ reduced analgesia by 71.6%, compared to Glu (P=0.02; d'=0.82, 95%CI 1.524-0.123). Aq$^+$ was superior to Glu at improving pain, KOOS subscales, physical function and analgesia use in mild-sKOA. Given these data, Aq$^+$ should be considered as a supplementary treatment for early-stage-KOA and may have the potential to reduce use of pain medication, although larger replication studies are required.

Keywords: Lithothamnion, pain management, nutraceutical, seawater magnesium

1. Introduction

Osteoarthritis (OA) is a pro-inflammatory condition of synovial joints that lead to significant morphological change [1]. Approximately 13% of over 50's suffer from symptomatic OA, with ~16-27% reporting Knee OA [KOA; 2, 3-7]. The reported incidence are estimated to rise with population age [7] and obesity rates [8] by ~2-4% annually, resulting in a lifetime risk of 61% in obese individuals [9, 10]. The symptoms include pain, stiffness, reduced range of motion and functional disability [11, 12] that can affect fatigue and psychological well-being

[13, 14]. The risk of 'mobility-disability' attributable to KOA is greater than any other medical condition in those >65 years [15-17] - with pain the most common complaint [18] and some degree of movement limitation [17]. Importantly, structural progression is not well correlated with pain [19], thus it is important to consider treatments for both symptomatic and radiographic KOA.

KOA is a progressive condition with no cure where acetaminophen and non-steroidal anti-inflammatory drugs (NSAID) are the traditional, non-lifestyle, approach for early clinical management. However, NSAIDs in particular have harmful side effects such as severe cardiovascular events [20]. Therefore, non-pharmaceutical alternatives exist and are recommended as early treatment [21-23] to improve symptoms [24-27]. Glucosamine (Glu) preparations are the most widely used nutraceutical for OA, with a global market value of ~1.03 billion USD by 2025 (www.businesswire.com, 2016). Despite large consumer investment, there is little consensus on the efficacy of Glu to treat OA [21, 28-33]. In a recent Cochrane review (25 RCTs), Glu failed to show any benefit for pain [34, updated 2009] and in a meta-analysis, re-examining individual patient data Glu was not superior to placebo for reducing knee pain [35]. However this report has been debated by proponents of particular Glu preparations [36] that have been recommended by some [37]. Nonetheless, current recommendations from the American College of Rheumatology [38] and others [39] do not support any use of Glu. Regardless, while Glu compounds have a lower risk of adverse effects than NSAIDs and other commonly used treatments, Glu shows higher rates than placebo [31]. Therefore, alternative over-the-counter nutraceuticals, without the side effects of NSAID and Glu, that show consist efficacy for improving KOA symptoms are required.

Lithothamnion species are rich in calcium, magnesium (Mg) and a variety of trace elements absorbed from sea-water during the organisms life [40]. Mineral-rich 'fronds' break off from the living organism, fall to the ocean floor and are harvested (Aquamin). The mineral extract contains ~30% calcium, ~2% magnesium, measurable levels of 72 other trace minerals [41] and has been shown to improve symptoms of moderate-to-severe KOA. Two double-blinded randomised trials utilising Aquimin F [AqF; 42, 43] improved KOA pain, symptoms (stiffness) and functional performance (6MWD) greater than Glu [43] and improved physical performance when NSAID use

was intentionally reduced by ~50% [42]. It remains to be seen if the positive effect of AqF on moderate-to-severe KOA are also present in mild-sKOA or if the treatment itself can reduce *ad libitum* medication use (rather than forced reduction).

Furthermore, deficiencies in Mg intake have been associated with KOA pain [44] and low serum Mg with radiographic severity [45], likely due to a greater Mg requirement in OA joints [46]. Data from animal and *in vitro* experiments suggest that Mg might improve pain and other symptoms, partly through neuropathic and nociceptive inflammatory mediators [47] - as Mg has a known role in nociceptive pain signalling pathways [48]. This may be important as recent OA studies have identified the inclement peripheral and central nerve sensitization [49], as well as nerve ending damage and regrowth [50, 51] in OA pain - thus the description of OA as a chronic mild-to-moderate nociceptive pain condition [52].

Additionally, Pine bark acts as a local anti-inflammatory in synovial fluid [53] and three recent publications have shown it to improve KOA pain and stiffness, NSAID use, physical and emotional well-being [54-56]. Pine bark preparations have recently been "strongly recommended" to the rheumatology community as early and additive treatment for OA, likely based on the most recent meta-analysis [21, 22]. Furthermore, the combination of nutraceutical compounds (such as those mentioned here) to optimise synergistic effects to improve OA symptoms has been suggested [23]. Therefore, the primary aim was to investigate the synergistic effects of *Lithothamnion species*, seawater-derived Mg and pine bark to reduce mild-knee pain, symptoms and physical function in sKOA, compared to Glu.

2. Materials and methods

2.1. Study design

In a double-blinded crossover pilot-trial of mild-sKOA (clinicaltrials.gov, NCT03106584), participants were randomly assigned (sequentially and concealed until intervention allocation) to either Glu or Aquamin$^+$ (Aq$^+$; details below) for 12 weeks. Following a 4 week washout period, participants transitioned to the opposite

supplement for an additional 12 weeks. Randomised allocation and intervention assignment was carried out by SH using Excel 'Rand' function, stratified by sex. All researchers and participants were fully blinded until after the statistical analysis was carried out. Unbinding responsibilities were allocated to a third party that was not involved in any element of the study. The primary outcome was pain, secondary outcomes were symptoms, physical function and analgesia use. There parameters were assessed pre and post intervention. The study was performed at the University College Dublin, Institute for Sport and Health between April 2017-October 2018. The study was conducted in accordance with the declaration of Helsinki (2013), was approved by the local University ethics committee and all participants gave informed written consent.

2.2. Participants

Participants were included if they reported mild-to-moderate knee pain, determined as Knee Injury and Osteoarthritis Outcome Score (KOOS) pain score of $\geq$50 [57]; aged 50-70 years and a BMI <35 kg/m^2. Participants were excluded if diagnosed with or suffered from osteoarthritic pain in any other lower body joints, rheumatoid arthritis, surgery in affected limb, injection or other non-pharmacological therapies within the last 12 months, any muscle disorder (such as sarcopenia etc.), serious medical comorbidities (such as arthrosclerosis, irritable bowel disease etc.), thyroid dysfunction or specific allergies.

Participants underwent X-ray radiography to determine the degree structural OA for participant characterisation and as recommended by the Osteoarthritis Research Society International [OARSI; 52, 58]. Both knees were X-rayed in a weight-bearing, semi-flexed position (~10-15 degrees) using a posterior-anterior beam direction (film focus distance 110 cm, 60 kV and 10 mA) with the aid of fluoroscopy to optimally align the tibia plateau. An independent consultant radiologist (SE) specializing in musculoskeletal radiology, blinded to the clinical data assessed joint space narrowing and osteophytes. To approximate Kellgren and Lawrence (KL) grade 1 or worse, one or more of the following criteria were fulfilled in either the medial or lateral tibiofemoral compartment: joint space narrowing grade 1 or worse, the sum of marginal osteophyte grades in the same compartment 1 or worse.

Three hundred and fifty-eight participants responded to the initial recruitment call and completed a secure online questionnaire to determine broad eligibility (questions included age, estimated height and mass, diagnosed medical conditions, medication or supplement use etc.). Eighty-two participants fulfilled the inclusion criteria (excluding n=276) and were then stratified by sex, and randomly selected for interview by phone, or in person, to ascertain specific inclusion (Inclusion and exclusion criteria detailed below) to achieve the recruitment target of thirty participants, determined by *a priori* power calculation (Fig 1).

2.3. Intervention

Participants consumed four capsules daily, two in the morning and two in the evening (with food), of either Glucosamine sulphate (Glu) or a combination of mineral rich algae (Aquamin) with seawater-derived $Mg(OH)_2$ [40] and pine bark *extract* (Aq$^+$). Each Glu capsule contained 500mg of Glu Sulphate and microcrystalline cellulose as a bulking agent (total daily dose 2000mg). Each Aq$^+$ capsule contained 667mg of milled mineral rich algae (Food and Drug Administration (FDA) recognized as safe (GRAS) 000028) with 2.5μg of vitamin D3 (Cholecalciferol) for mineral absorption, 67mg of seawater-derived $Mg(OH)_2$, 30mg of pine bark (*Pinus radiate;* containing >80% proanthocyanidins, >1% dihydropuercetin and other watersoluble flavonoids, flavonoid conjugates, and phenolic acids) and microcrystalline cellulose as a bulking agent (total daily dose; 2668mg mineral rich algae, 268mg $Mg(OH)_2$ and 120mg *Pinus radiate*). The Glu capsules were prepared by Nutrition Group (Nutrition Group PLC, Blackpool, UK). The Aq$^+$ components of mineral rich algae (Aquamin) and $Mg(OH)_2$ were provided by Marigot Ltd. (Marigot Ltd, Cork, Ireland). The pine bark was manufactured by Enzogenol® (ENZO Nutraceuticals Ltd., Paeroa, NZ) and the combination was prepared by Nutrition Group (Nutrition Group PLC, Blackpool, UK). All capsules were ivory coloured, indistinguishable between supplements and packaged in identical sealed screw top containers labelled as "A" or "B". Participant were provided the full 12-week supply upon baseline testing of each trial arm. Investigators and participants were blinded to the content of "A" and "B" until after the data analysis.

2.4. Treatment diary

During each arm, participants were given a detailed treatment diary consisting of intervention dosage, recording capsule consumption and medication use. Specifically, participants were asked to record the date and time of each capsule consumption, analgesia use (quantity of pills/gel used, name of medication and volume) and any adverse or positive side effects. Capsule consumption record was used to assess supplement adherence and quantification of analgesia use [similar to other pain designs; 59]. For the calculation of analgesia use, each pill/gel was given the value of "1", regardless of specific pharmaceutical drug (retrieval of specific medication volumes was not sufficient for analysis). Non-steroidal anti-inflammatory drugs (NSAID) were also evaluated.

2.5. Knee Injury and Osteoarthritis Outcome Score (KOOS)

For assessment of pain and symptoms, the well validated [60, 61] KOOS questionnaire was used [57]. The KOOS Pain Scale was chosen over the WOMAC because KOOS Pain has 4 additional items allowing for a more comprehensive assessment of pain with additional, clinically important, activity constructs. The KOOS consists of 42 items on 5 dimensions (pain, symptoms, activities of daily living (ADL), sport and recreational activity and quality of life) concerning the last 7 days [57]. A Likert scale was used, scored from 0 (no problems) to 4 (extreme problems) and each of the five dimensions were calculated as the sum of the items. Scores were transformed to a 0–100 scale, with zero representing extreme and 100 representing no knee problems [57]. Each dimensions was assessed separately and the Minimally Clinically Important Difference (MCID) was 8 units for all KOOS dimensions.

2.6. Physical Activity Scale for the Elderly (PASE)

PASE was used to assess recent physical activity [62, 63] with the 12 items in three dimensions of leisure activity, household activity and work related activity. The frequency, duration, and intensity level of activity over the previous 7 days were used to assign a score, ranging from 0 to 793. A higher total score, represents greater achieved physical activity.

2.7. Anthropometrics

Height and Mass were measured by standard methods and were used to calculate BMI ($kg \cdot m^2$). Dual Energy X-Ray Absorptiometry (DEXA; Lunar iDXA; GE Healthcare, Buckinghamshire, UK) was performed to measure body composition following a 12 h overnight fast. Participants lay in a supine position, avoiding contact between the trunk and the appendicular mass during the procedure (effective dose, <6 μSv). Participants were asked to remain completely still for the duration of the scan. To ensure measurement accuracy and reliability, the DXA scanner was 'quality assured' before each test session (densitometry block supplied by the manufacturers). All scanning and subsequent analyses procedures was undertaken by trained DEXA operators.

2.8. Functional performance

2.9.1 Timed up and Go (TuG)

The TuG was performed using a standard high-back adjustable orthopaedic armchair, set at seat height 46cm and arm height 67cm [64, 65]. Participants sat against the back of the seat, feet maintaining full contact with the floor and were instructed to rise from without the aid of the armrests and walk three metres from the anterior surface of the arm chair legs and return to the start position. Participants were timed from when their buttocks left the chair until their buttocks returned to the chair. TuG test was repeated three times and an average value was calculated. Participant were instructed to use their "comfortable and safe walking speed". Faster time represent better functional performance.

2.9.2 Six Minute Walk Distance (6MWD)

Participants performed the 6MWD, often used in arthritic and elderly populations [65-67]. A 25 metre course was laid out with both ends visibly marked. Participants began walking with the instruction to "walk at their regular comfortable walking pace" for six minutes. During the test, participants were given verbal encouragement and their maximal distance over the six minute period was recorded.

2.9. Statistical analysis

A priori Power calculations determined the required sample size plus ~10% for predicted drop-outs, given the primary outcome of KOOS pain pre and post intervention (effect size 0.4 and 1-β 0.8 at a Type 1 error of 5%; G* Power). As this was an exploratory pilot-trial and with recommendations to identify sex subgroups [68] the data were analysed as the entire group (sKOA) and by sex. Non-parametric Wilcoxon Signed Rank Tests were used for comparisons of all KOOS variables and analgesic use in sKOA; for males, ADL and TuG; for females, Symptoms, ADL, QoL and TuG (not nominal distributed, Kolmogorov-Smirnov). For all other variables two-tailed pared-sample T-tests were used to compare the within-group effects. Cohens *d'* was used to estimate the effects (https://www.psychometrica.de/effect_size.html). Pearson's or Spearman's correlations coefficients were used to assess relationships between selected variables. Assess the assumption of negligible carryover effect and between-group inferiority, the Wellek-Blettner method [69] with independent-sample T-tests were carried out. Null hypothesis and Pearson statistics were performed using SPSS 24 (SPSS Inc., Chicago, IL) with alpha set at P=0.05.

3. Results

One participant discontinued the Aq+ arm, citing gastrointestinal discomfort (this was the only reported adverse event), but completed the Glu arm. One additional participant did not complete the functional performance assessments (TuG and 6MWD) citing personal scheduling conflict (Fig 1). Participant characteristics, PASE, Kelgren-Lawrence grade and knee pain history are presented in table 1. The supplements were well tolerated, resulting in 93% treatment adherence, measured by patient reported treatment diary logs and pill count. There was no evidence of pre-test carryover between crossover arms for any variables (P range, 0.53-0.98) or using the Wellek-Blettner method (P value range, 0.07-0.69). Additionally, Wellek-Blettner between-group effect showed no violation of inferiority between the two treatments (P>0.68).

3.1. Primary outcome

3.1.1 KOOS pain

For sKOA (i.e. the whole sample), only Aq$^+$ improved pain (Aq$^+$, d'=0.73, P<0.01), and exceeded the MCID (Fig 2). When separated by sex, Aq$^+$ improved pain in both males and females (P<0.01), with the greatest effect in males (d'=0.91), compared to females (d'=0.55). Glu did not improve pain (P>0.05; Table 3).

3.2. Secondary outcomes

3.2.1. Symptoms

Aq$^+$ improved symptoms for sKOA and females (P<0.02) but had no effect in males (P>0.05). There was no effect of Glu (Table 2 and 3).

3.2.2. Activities of daily living (ADL)

Glu improved ADL in sKOA (P=0.02),with no effect of Aq$^+$ (P>0.05; Table 2 and 3).

3.2.3. Sport and recreation

Aq$^+$ improved sport and recreation (P=0.02) for sKOA and females, with females exceeding the MCID and no effect of Glu (Table 2 and 3).

3.2.4. Quality of life (QoL)

There was no change in KOOS QoL for either Aq$^+$ or Glu (P>0.05; Table 2).

3.2.5. Physical Activity Scale for the Elderly (PASE)

There was no change in PASE for either Aq$^+$ or Glu (P>0.05; Table 2).

3.3. Function performance outcomes

There was no change in 6MWD for either supplement or sex (P>0.05). In females, Aq$^+$ improved TuG performance by -0.43s (P=0.04, d'=0.43) and Glu worsened by 0.24s (P=0.27). Aq$^+$ improved TuG percentage

change by -7.02% (d'=0.92) while Glu worsened by 4.18% (P=0.04, Table 2 and Fig 2). There was no change in TuG for either supplement in males (P>0.26).

There was a modest inverse correlation between improvements in pain and TuG performance for females irrespective of supplementation (r=-042.; R^2=0.17; P=0.01; Fig 3). However, this was a product of the Aq^+ (r=-0.49; R^2=0.24; P=0.04; Fig 3) rather than Glu (P>0.08).

3.4. Analgesia use

Forty-three (of 59) treatment diaries were retrieved with adequate information, 34 (17 each for Aq^+ and Glu) reported analgesic medication to manage knee pain during the trial period (Fig 5). Reported analgesics were; Paracetamol, Brupro, Panadol, Neurofen, Voltare, Asprin, Solpadene, Bluplex, Ibuprofen, Vimovo, Arcoxia, Voltarol gel and Difene spray. Participants reported using significantly more analgesics during the Glu arm of the study compared to Aq^+ with (72%; P=0.03; d'=0.82). Of the reported analgesics that were use, 35% were NSAID and participants consuming 65% more NSAID during the Glu compared to Aq^+ (P=0.07; d'=0.38).

After removal of outliers (≥ three standard deviations above the mean; n=30 data points included), greater analgesic use was correlated with lower TuG performance (r=0.43, R^2=0.18, P=0.02). This correlation was driven by a stronger relationship for greater analgesia use with worsening TuG performance during the Glu arm (r=0.60, R^2=0.36, P=0.02).

4. Discussion

This pilot-trial has shown that the combination of *Lithothamnion species*, seawater-Mg and pine bark (Aq^+) effectively improved pain in mild-symptomatic osteoarthritis. Specifically, Aq^+ reduced pain beyond the MCID in the whole cohort, in both sexes independently, and was superior to Glu. In females, functional performance (TuG) improved only in Aq^+ and this improvement was correlated with reduced pain. Of particular interest, *ad libitum* use of analgesic medication was 72% lower during the Aq^+ arm compared to the Glu.

The present data show no improvements in pain for Glu which is similar to other Glu and KOOS findings [70], and the general consensus on the efficacy of Glu. In contrast, Aq⁺ had a large effect on improving pain and exceeded the MCID in 10% more individuals, compared to Glu (44% and 53% respectively). When sex was considered independently, Aq⁺ improved pain to the greatest extent in males (d'=0.91), compared to no effect with Glu. This is not surprising as OA subpopulations (sex), can have significant impacts on study results [71, 72]. These data are consistent with previous findings in symptomatically worse (moderate-to-severe) sKOA treated with *Lithothamnion species* [AqF; 43]. Frestdt et al. [42] showed that AqF improved pain, with no difference in placebo or when Glu and AqF were combined. The present study, using an alternative but well validated assessment of symptomatic OA [KOOS; 60, 61], advanced these findings, showing improvements for pain and KOOS subscales in mild-sKOA treated with Aq⁺. These advances are possibly due to the addition of seawater-derived Mg and pine bark. In previous studies, pine bark extract reduced pain by ~40% [55, 56], whereas lower Mg intake was associated with worse KOOS and WOMAC pain [1.5 points for every 50mg; 44]. Furthermore, a single dose of inter-articular Mg following arthroscopic surgery reduced postoperative pain and increased time-to-analgesic use [73].

Frestdt et al. [42] further showed that AqF improved physical function (6MWD, 8.7%) after eight weeks of treatment. This improvement was likely a result of reduced pain although the authors did not quantify a possible relationship [42]. The present results also showed improvements in physical function through the TuG, but not with walking distance. There was an inverse correlation between improvements in pain and physical function in females irrespective of treatment, but only in Aq⁺ when separated by intervention. However, it must be noted that the present results, while null-hypothesis significant, represent a limited relationship and need replication (Aq⁺ - Females, R^2=0.24; Fig 4). These data relate to a relatively young (~60y), active mild-sKOA population and is a welcome advancement considering that KOA patients have the greatest risk of mobility-disability than any other medical condition in people aged >65 years [15, 16]. KOA is strongly associated with fatigue [13], joint instability [74], frailty [75] and long-term cardiovascular disease [76] and may suffer from the symptoms of OA

for more than three decades [77]. Considerable attention should be given to any treatment that can reduce the decline in functional capacity and potential fall-risk, while improving quality of life in 'years lived with disability' in those with OA [78].

There are several possible mechanisms for the present results, for example the increased Mg and Calcium (Ca^+) intake during Aq^+, as lower serum Mg and Ca^+ are evident in OA and this is inversely related to worse KOA [45, 79, 80]. A recent longitudinal study showed that, not achieving the recommended daily intake of Mg increases your frailty risk (by proxy, reduced physical function) up to 51% [75] and was further verified with low Mg intake in those with greater pain and worse function in the same cohort - likely due to inflammatory mediation by Mg [44]. This relationship has a strong molecular rationale [47], however the present data are the only that exist showing orally supplementing Mg (albeit as part of a mineral combination) may improve function and symptoms in KOA. Following the role of inflammatory mediating nutraceuticals, pine bark extracts have shown to improve treadmill walking distance by >100m compared to placebo [54] and is substantiated with other measures of physical function [53]. The combination of these nutraceutical components could potentially enhance the synergistically positive benefits on physical function evident in the present investigation, advancing those of *Lithothamnion species* without these added compounds [42].

Of particularly note, during the Aq^+ arm the use of analgesics was 72% and NSAIDs were 65% less than with Glu. While we did not assess analgesia/NSAID use before and after each intervention (simply usage during the trial), our data are in line with others that have shown no reduction analgesia/NSAID use with Glu [81]. Any reduction in NSAID or analgesics is important as recent large-scale analysis of NSAIDs and risk of heart failure-hospital admissions showed 1.83 greater odds [Ketorolac; 82]. In a randomised double-blinded trial of OA and rheumatoid arthritis patients (n=24,081), the commonly used NSAID 'ibuprofen' (prevalent in the present cohort) had the highest rate of NSAID toxicity (HR=1.38), closely followed by naproxen [83]. The present result of reduced analgesics may be, in part, driven by the inclusion of the 150mg of pine bark extract in Aq^+ [54]. In early-stage KOA, pine bark extract in isolation reduced NSAID usage by 58%, compared to a 1% decrease in placebo

[54]. This is similar to the present findings of reduced NSAID use (65%) but less than the 72% reduction in all analgesia usage. It can be inferred that the combination of *Lithothamnion species*, seawater-Mg [noting the effect of inter-articular Mg on time-to-analgesic use; 73] with pine bark might aided in the further reduction of analgesic use. Furthermore, a previous report showed superior improvements in physical function during a period of 50% 'forced reduction' from NSAID use with AqF [42]. In agreement with this finding, the present results identified a modest correlation for increased medication use with worsening TuG performance (r=-0.43, R^2=0.18), driven by worse performance with Glu (r=0.60, R^2=0.36). It is possible that the mineral composition of *Lithothamnion species* (particularly Ca^+) was a main driver [42, 79], but also the additional seawater-Mg [40] and pine bark [53]. A number of Mg-associated pain mediating molecular mechanisms have recently been proposed [47] with reduced serum Ca^+ inversely correlated with KOA severity [79] and pine bark, previously shown to improve both medication use and physical function [54].

There are limitations to the present study that need to be highlighted although every effort was made to mitigate their effects. The present sample included a relatively small sample [although similar to other nutraceutical designs; 54, 56, 84, 85] however, the crossover design mitigated some of this limitation and controlled individual variation, as participants completed both arms of the trial. Assessment of dietary minerals intake/status was not considered in the present trial but would allow the estimation of mineral deficiencies. Nonetheless, participants were instructed not to alter their diet during the trial and were questioned as such during laboratory visits. The current investigation did not include a placebo; however the aim of the study was to directly compare the efficacy of the market leading over-the-counter treatment for OA and an alternative natural complex, therefore a placebo was not strictly required for this pilot-design. Finally, the present sample included only mild-sKOA and as such the conclusions can only be generalised to this population. However, it is likely that similar effects (although altered magnitudes) would be evident in moderate-to-severe sKOA.

In conclusion, the combination of *Lithothamnion species*, seawater-Mg and pine bark (Aq^+) was superior to Glu at improving pain, KOOS subscales, physical function and analgesia use in those with sKOA. These results were

consistent across sex and provide evidence for an effective treatment to improve tangible symptoms of sKOA

pain. Furthermore, as Aq$^+$ reduced analgesia use, it could be considered an effective treatment for mild-sKOA

both independently and/or in combination with other pharmaceutical or non- pharmaceutical treatments.

5. Conflict of interest statement

Authors contributions

GDV and ED designed the initial study concept and GDV, ED and SH refined the study design. CM, SE and GDV provided medical consultations, advice and specialized assessment. SH and RF conducted the trial, collected and processed the data. SH and ED performed the statistical analysis. SH drafted the initial manuscript, data presentation and all authors were involved in subsequent drafting and approval of the final manuscript.

6. Acknowledgements

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Figure legends

**Figure 1** Knee pain participant recruitment, selection and completion flow diagram.
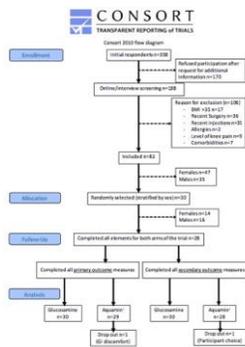
Figure 2 Change in TuG performance for Aq$^+$ (black bars) and Glu (grey bars). Panel **A** presents individual

percentage change data for females, mean percentage change with standard deviation error bars (top insert)

and mean absolute change with standard deviation error bars (bottom insert). Dollar sign indicated null-

hypothesis significance accompanied by effect estimate (Cohens *d'*).

Figure 3 Correlations coefficients for changes in TuG performance with KOOS pain for all females (panel **A**). Black

dots and trendline are Aq$^+$, grey dots and solid grey trendline are Glu. Dashed grey trendline represents all data.
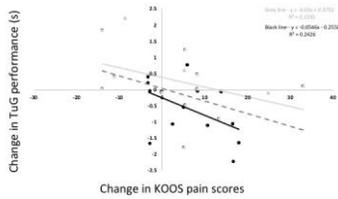
Figure 4 Individual participant analgesia use during the Glu arm (grey bars) and the Aq$^+$ arm (black bars). Mean analgesia use for both Glu and Aq$^+$ (insert). Dollar sign indicated null hypothesis significance accompanied by effect estimate (Cohens *d'*).
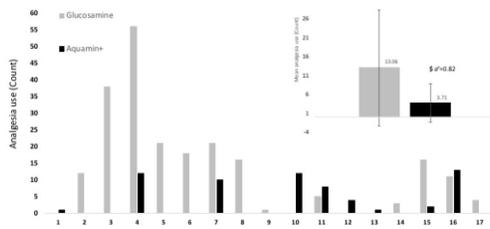
Table 1 Participant characteristics, presented as means ± standard deviations.

	Males (n=16)	Females (n=14)	All (n=30)
Years with $_S$KOA	15.4 ± 9.4	5.4 ± 4.3	10.6 ± 9.1
Age (y)	61.4 ± 4.4	60.3 ± 5.9	60.9 ± 5.1
Height (m)	1.82 ± 0.07	1.65 ± 0.06	1.74 ± 0.11
Mass (kg)	86.2 ± 9.4	71.0 ± 10.5	79.1 ± 12.4
BMI (kg·m^2)	26.0 ± 1.9	26.0 ± 3.3	26.0 ± 2.7
Body fat (%)	27.3 ± 6.0	39.1 ± 5.8	32.8 ± 8.4
Lean muscle mass (kg)	59.8 ± 7.0	41.1 ± 3.9	51.1 ± 11.1
Total BMD (g·cm^2)	1.255 ± 0.149	1.116 ±0.099	1.190 ± 0.144
KL grade (n)			
0	5	4	9
1	7	8	15
2	0	2	2
3	0	0	0
4	4	0	4

BMI, body mass index; BMD, bone mineral density; KL, kellgren-lawrence.

Table 2 Participant data pre and post intervention for both trial arms, presented as means ± standard deviations.

			Males (n=16)		Females (n=14)		All (n=30)	
			Pre	Post	Pre	Post	Pre	Post
KOOS								
	Pain							
		Glu	73.7 ± 15.7	80.0 ± 13.5	72.3 ± 16.3	67.8 ± 12.6	73.1 ± 15.7	78.5 ± 12.9
		Aq⁺	71.5 ± 10.2	81.8 ± 12.2 [# $]	69.4 ±10.9	75.6 ± 11.7 [#]	70.5 ± 10.4	78.8 ± 12.2 [# $]
	Symptoms							
		Glu	73.8 ± 21.7	78.8 ± 17.3	73.5 ± 16.7	75.8 ± 16.6	73.1 ± 19.2	77.7 ± 16.8
		Aq⁺	74.3 ± 15.3	79.5 ± 16.5	72.4 ± 18.4	77.8 ± 18.5 [#]	73.4 ± 16.6	78.7 ± 17.2 [#]
	ADL							
		Glu	81.0 ± 17.1	86.2 ± 14.3	76.5 ± 16.1	80.7 ± 15.9	78.9 ± 16.5	83.6 ± 15.1 [#]
		Aq⁺	83.2 ± 9.6	87.7 ± 13.3	76.2 ± 16.0	76.7 ± 18.8	79.8 ± 13.4	82.4 ± 16.9
	SR							
		Glu	74.1 ± 24.0	79.1 ± 20.4	67.0 ± 19.8	73.6 ± 18.0	70.8 ± 22.1	76.5 ± 19.2
		Aq⁺	74.0 ± 19.5	78.2 ± 22.8	62.5 ± 23.6	72.5 ± 23.8 [# $]	68.4 ± 22.0	75.4 ± 23.0 [#]
	QoL							
		Glu	59.8 ± 16.9	62.9 ± 14.2	58.6 ± 17.8	58.5 ± 19.1	59.3 ± 17.0	60.8 ± 16.5
		Aq⁺	60.1 ± 10.8	62.9 ± 17.0	56.8 ± 13.8	59.4 ± 19.9	58.5 ± 12.2	61.2 ± 18.2
PASE								
		Glu	197.6 ± 78.6	179.4 ± 70.0	215.0 ± 118.1	184.0 ± 124.1	205.7 ± 97.7	181.6 ± 97.2
		Aq⁺	184.0 ± 80.7	168.9 ± 95.8	210.6 ± 88.4	171.4 ± 62.6	196.8 ± 84.1	170.1 ± 83.9
TuG (s)								
		Glu	6.50 ± 1.36	6.81 ± 1.21	7.03 ± 1.57	7.27 ± 1.46	6.74 ± 1.41	7.02 ± 1.33
		Aq⁺	6.63 ± 1.39	6.84 ± 1.19	7.39 ± 1.45	6.80 ± 1.19 [#]	7.37 ± 2.72	6.82 ± 1.17
6MWD (m)								
		Glu	555.7 ± 71.0	566.3 ± 68.8	505.6 ± 77.9	491.1 ± 63.0	532.3 ± 77.3	531.2 ± 75.4
		Aq⁺	566.0 ± 81.6	560.9 ± 69.8	503.1 ± 77.0	535.9 ± 76.7	534.4 ± 82.7	548.4 ± 73.1

KOOS, knee injury and osteoarthritis outcome score; ADL, activities of daily living; SR, sport and recreation; QoL, quality of life; PASE, physical activity scale for the elderly; TuG, timed up and go; 6MWD, six minute walking distance.

[#]indicates null-hypothesis difference from pre-intervention.

[$]indicates a delta change beyond the MCID.

Table 3 Cohens *d'* with 95% confidence intervals for KOOS subscales and TuG mean change in sKOA, males and females that showed null-hypothesis differences.

		All		Males		Females	
		d'	95%CI	*d'*	95%CI	*d'*	95%CI
KOOS							
Pain							
	Aq⁺	0.73	0.201-1.265	0.91	0.162-1.667	0.55	0.210-1.299
Symptoms							
	Aq⁺	0.31	0.204-0.829	-	-	0.29	0.454-1.036
ADL							
	Glu	0.30	0.213-0.805	-	-	-	-
SR							
	Aq⁺	0.31	0.207-0.829	-	-	0.42	0.352-1.144
TuG							
	Aq⁺	-	-	-	-	0.45	1.195-0.305
Analgesia		0.82	1.524-0.123	-	-	-	-
***NSAID**		0.38	1.519-0.754	-	-	-	-

KOOS, knee injury and osteoarthritis outcome score; ADL, activities of daily living; SR, sport and recreation; TuG, timed up and go; NSAID, non-steroidal anti-inflammatory drugs. There was no significant association for KOOS quality of life and as such it was omitted from the table (all variables were P<0.05). *NSAID P=0.07.