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ORIGINAL RESEARCH

A combined phase I and II open label study on the effects of a seaweed extract nutrient complex on osteoarthritis

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Background: Isolated fucoidans from brown marine algae have been shown to have a range of anti-inflammatory effects.

Purpose: This present study tested a Maritech® extract formulation, containing a blend of extracts from three different species of brown algae, plus nutrients in an open label combined phase I and II pilot scale study to determine both acute safety and efficacy in osteoarthritis of the knee.

Patients and methods: Participants (n = 12, five females [mean age, 62 ± 11.06 years] and seven males [mean age, 57.14 ± 9.20 years]) with a confirmed diagnosis of osteoarthritis of the knee were randomized to either 100 mg (n = 5) or 1000 mg (n = 7) of a Maritech® extract formulation per day. The formulation contained Maritech® seaweed extract containing *Fucus vesiculosis* (85% w/w), *Macrocystis pyrifera* (10% w/w) and *Laminaria japonica* (5% w/w) plus vitamin B6, zinc and manganese. Primary outcome was the average comprehensive arthritis test (COAT) score which is comprised of four sub-scales: pain, stiffness, difficulty with physical activity and overall symptom severity measured weekly. Safety measures included full blood count, serum lipids, liver function tests, urea, creatinine and electrolytes determined at baseline and week 12. All adverse events were recorded.

Results: Eleven participants completed 12 weeks and one completed 10 weeks of the study. Using a multilevel linear model, the average COAT score was reduced by 18% for the 100 mg treatment and 52% for the 1000 mg dose at the end of the study. There was a clear dose response effect seen between the two treatments ($P \leq 0.0005$) on the average COAT score and each of the four COAT subscales (pain, stiffness, difficulty with physical activity and overall symptom severity) ($P \leq 0.05$). The preparation was well tolerated and the few adverse events were unlikely to be related to the study medication. There were no changes in blood parameters measured over the course of the study with the exception of an increase in serum albumin which was not clinically significant.

Conclusion: The seaweed extract nutrient complex when taken orally over twelve weeks decreased the symptoms of osteoarthritis in a dose-dependent manner. It was demonstrated to be safe to use over the study period at the doses tested. The efficacy of the preparation now needs to be demonstrated in a phase III randomized controlled trial (RCT).

Australian and New Zealand Clinical Trials Register: ACTRN12607000229471.

Keywords: fucoidan, osteoarthritis, complementary medicine, inflammation, TNF alpha

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Introduction

Osteoarthritis (OA) is the most frequent cause of disability among adults in the developed world. Arthritis affects around three million people in Australia, representing about 15% of the population.[1] Similarly, more than 20 million people in the United States have the disease.[2] The lifetime risk of knee OA for males and females aged over

45 years in Johnston County is estimated at between 44.7% (nonobese) and 67% (obese) and these figures are believed to reflect the incidence of OA across the United States.[3] OA costs more than $60 billion per year in the USA and is second only to ischemic heart disease as a cause of work disability in men aged over 50 years.[4] The progressive deterioration of articular cartilage which occurs in OA results in pain, stiffness and difficulty with physical activities. The disease is managed rather than cured, with a focus on pain relief.[5] A number of herbal medicines have been found to have beneficial effects in alleviating the symptoms of OA in human clinical studies. These include advocado, soybean unsaponifiables,[6] lipids from green-lipped mussels,[7] calcified seaweed extracts,[8] and Pycnogenol (French maritime pine bark extract).[9] *Boswellia serrata* extracts have also show clinical promise[10] as do preparations of *Harpagophytum procumbens* (Devil's Claw).[11,12] Polyphenols such as epigallocatetchin (from green tea) and phlorotannin-rich extracts of the seaweed Ecklonia cava have exhibited potential using *in vitro* models yet have generated little clinical evidence to date.[13]

In the Western herbal medicine tradition, bladderwrack (*Fucus vesiculosus*) and other seaweeds in the form of topically applied liniments have been used as herbal approaches to the treatment of sore knees.[14] Seaweed extracts have been demonstrated to contain at least two major components with anti-inflammatory activity; fucoidans and polyphloroglucinols (algal polyphenols). Fucoidans are considered to be one of the main therapeutic components of brown algae[15] and may constitute up to 25%–30% of the algal dry weight, depending on the specific seaweed species.[16] Although fucoidans are highly branched long chain polysaccharides, low levels of serum uptake of fucoidan were observed after Undaria fucoidan ingestion[17] indicating that there is potential for clinical activity. Fucoidan is a potent selectin blocker and has been used experimentally to prevent inflammatory damage after ischemic events.[18,19]

Polyphenolic fractions derived from seaweed also have profound antioxidant activity[20] which can also contribute to anti-inflammatory effects. Inhibition of oxalate damage to kidneys in animal models was attributed to the antioxidant qualities of *Fucus*-derived fucoidan.[21] Fucoidans have been shown to inhibit phospholipase A_2[22] an important enzyme in the inflammatory cascade inhibited by corticosteroids.

The toxicity of seaweed extracts has been investigated in both human and animal studies, although the source of the extracts has been different to the Fucus source used in this study. Previous human clinical studies using 3 g daily Undaria seaweed extracts with 75% fucoidan content indicated no clinically observed toxicity.[23] In a recent companion study on the coagulation effects of 3 g of the same Undaria extract, there was a significant change in clotting indices, but these remained within clinically normal parameters. Activated partial thromboplastin time increased from 28.41 to 34.01 s (n = 10; $P = 0.01$), thrombin time decreased from 18.62 to 17.55 s (n = 10, $P = 0.04$), and anti-thrombin-III increased from 113.5% to 117% (n = 10, $P = 0.03$).[24] There were no toxicological changes observed in rats given up to 300 mg/kg orally of fucoidan from *Laminaria japonica*. This dose is considerably higher that the 3000 mg per subject dose in the clinical studies. The absence of observations may be because of difference in the source species for the fucoidan, or the absorption of the fucoidan from the gut. Anticoagulant effects were observed at doses of 900 to 2,500 mg/kg, but no other signs of toxicity were observed.[25] In a similar study involving fucoidan extracted from Cladosiphon okamuranus, no significant toxicological changes were induced by fucoidan at a dose of 600 mg/kg of body weight/day in Wistar rats. However, with concentrations at and above 1,200 mg/kg of body weight/day, clotting time was significantly prolonged.[26] Overall, the dose levels used in this study (a total of either 100 mg and 1000 mg per day) would not be expected to produce changes outside of the clinically normal parameters, as they are many fold lower than the dose levels used in the animal studies, and a third of the dose used in the human clinical study. A polyphenol rich fraction of *Fucus* was also shown to lack acute toxic effects in rats after four weeks of oral dosing.[20] The latter extract was somewhat different to the extract used in this study as it contained a concentrated polyphenol fraction.

The present study tested a seaweed extract nutrient complex containing a blend of extracts from three different species of brown algae plus nutrients zinc, manganese and B6, using an open label design at two doses to determine effects in OA. This was a pilot scale combined phase I and II study aimed at providing data on acute safety and efficacy.

Material and methods

Research design

This trial was a pilot scale open label dosing study, with two different doses randomised to participants, conducted over 12 weeks (84 days) in Lismore New South Wales (Australia) and was conducted in 2008. The study was approved by the Human Research Ethics Committee of Southern Cross University (Ethics approval number: ECN-07-36). The research was conducted in compliance with Good Clinical

Practices (GCP) and in accordance with the guidelines of the Australian National Health and Medical Research Council and the Declaration of Helsinki (as revised in 2004). The trial was registered with the Australian and New Zealand Clinical Trials Register (ACTRN12607000229471).

Participants

A convenience sample of healthy individuals aged between 18 and 65 years was recruited by email from staff and students at Southern Cross University, and from Lismore and surrounding areas through newspaper advertising, regional radio and television. All participants received a study information sheet outlining the study and signed an informed consent form agreeing to participate.

Participants were included if they had both X-ray and clinical evidence of osteoarthritis of the knees; if they had a baseline comprehensive osteoarthritis test (COAT) score between 3 and 7; were otherwise healthy (had no other acute nor chronic medical condition); and if they were willing to discontinue their current OA treatment for the duration of the study. Participants were excluded if they had a history of trauma associated with the affected joint; if they had rheumatoid arthritis or other inflammatory joint conditions (including gout); if they used corticosteroids (intra-articular or systemic) within four weeks prior to baseline and throughout the study; if they used anti-inflammatory agents or anti-arthritic complementary medicines three weeks prior to baseline and during the duration of the study; had liver function tests greater than three times the upper limit of normal at baseline; if they had a history of alcohol or substance abuse; were female participants who were lactating, pregnant or planning to become pregnant; if they had participated in another clinical trial in the last 30 days; if they were unwilling to have blood taken three times during the study; or if they were unwilling to comply with the study protocols.

Outcome measurements

The primary outcome measurement in this study were the safety measures and the average COAT score, a validated measurement instrument for the assessment of the symptoms of osteoarthritis.[27] The COAT score is composed of four subscales: pain, stiffness, difficulty with physical activity and overall symptom severity. Secondary outcomes included the individual COAT sub-scales, TNF-alpha measurements, serum lipids and paracetamol usage over the 12 week study.

Baseline COAT measures were recorded and participants with visual analog scale (VAS) scores between 3 and 7 out of a possible 10 who agreed to wash out from their current osteoarthritis treatment were admitted to the study. Wash out commenced four weeks prior to the start of the trial and participants did not take their current osteoarthritis medications for the duration of the study. The participants were supplied with study diaries and asked to record their COAT scores and paracetamol use daily for four weeks. They were requested not to take narcotic analgesics and those containing codeine for seven days before the next clinic and until the end of the study. Study staff contacted the participants each week at the same time to ensure compliance and provide support.

COAT scores included joint pain, stiffness, difficulty with physical activities and overall symptom score. The participants completed a baseline COAT score under the supervision of the clinic staff by making a mark on a 10 cm VAS with a single vertical line to show the severity of each descriptor over the past twenty-four hours. The descriptors were none to extreme with a numerical score of 0 to 10. The scores were converted to a numerical grade through measurement with a ruler placed on the mark. Daily diaries were issued along with identical rulers (manufactured by Celco) and study staff contacted the participants each day at the same time to collect scores, ensure compliance and provide support. These instructions complied with the methods used with the validated tool. Participants were screened four weeks prior to the trial, they returned two weeks prior to ensure that scores remained between 3 and 7, and then returned at baseline. If the scores remained between 3 and 7 at baseline, the participant was randomised to a study medication.

Safety was assessed by actively monitoring adverse events. Subjects were questioned weekly about adverse events which were then recorded. Participants attended three clinics during which weight, blood pressure, pulse rate, and concomitant medication use was recorded and fasting blood samples collected at baseline, week 4, and week 12. Safety measurements undertaken by an independent accredited laboratory were full blood count, liver function tests and determination of urea, creatinine, electrolytes, cholesterol and triglyceride concentrations to assess toxicity to the hemopoietic, hepatic and renal systems; and assess the impact on fasting lipids. The body mass index (BMI) was calculated at the start and conclusion of the study.

Subjects returned all remaining capsules at each clinic visit and these were counted as a measure of compliance. The investigator maintained an inventory record of all capsules received and dispensed. It was assumed that capsules not returned were taken.

Participants were asked at the end of the study to evaluate the study medication by answering a question indicating their satisfaction, dissatisfaction, or neither satisfaction nor dissatisfaction with the study medication.

Study medication and dose

The study medication used Maritech® extract (Marinova Pty Ltd, Hobart, Australia). These are fucoidan-rich extracts of seaweeds (Maritech® extracts) which are manufactured using a proprietary aqueous process that produces fucoidan fractions. Nothing is added during the process. Insoluble matter and salts are removed as part of the process. They are 'whole plant' extracts which contain fucoidan and seaweed polyphenols (polyphloroglucinols) associated with the fucoidan molecule. In this study we used a blend of three Maritech® extracts from different species of brown seaweeds; Maritech® *Fucus vesiculosis* (85% w/w), Maritech® *Macrocystis pyrifera* (10% w/w) and Maritech® *Laminaria japonica* (5% w/w). In addition, Vitamin B6, zinc sulfate and manganese sulfate were included in the formulation, as detailed in Table 1. The study medication was manufactured as 100 mg and 250 mg capsules (Gel Caps) under the code of good manufacturing practice (GMP). The total fucoidan concentration in the 100 mg and 250 mg capsules was 75 mg and 187.5 mg respectively. Fucoidan content is assessed using a validated spectrophotometric method based on a modified Dubois method.[28]

Subjects were randomized to either a100 mg or 1000 mg dose group using the research randomizer website (http://www.randomizer.org/). Seven sets of two numbers per set, using a range of one to two, were generated. These numbers were used to assign the participants to either the 100 mg or 1000 mg dose. The 100 mg dose was delivered in one 100 mg gel capsule daily (taken in the morning) to five participants. The 1000 mg dose was delivered as four 250 mg capsules daily (two capsules taken twice daily) to seven participants. The capsules were self-administered orally by the participants after food.

Table 1 Contents of Maritech® capsules

Component	100 mg	250 mg
Maritech® *Fucus vesiculosis*	85 mg	212.5 mg
Maritech® *Macrocystis pyrifera*	10 mg	25 mg
Maritech® *Laminaria japonica*	5 mg	12.5 mg
Pyridoxine hydrochloride	57.50 mg	14.38 mg
Zinc sulphate monohydrate	25 mg	6.25 mg
Manganese sulphate	4 mg	1 mg

TNF alpha assays

After collection serum was stored at −70 °C until analyzed. Serum tumor necrosis factor-α (TNF-α) was measured using a sandwich ELISA assay (Cayman Chemical Company, Denver, CO, USA). Thawed serum was supplemented with 5% mouse serum and 9 mM dithiothreitol in order to minimise nonspecific binding to the mouse anti-TNF-α. Serum was assayed without further dilution. TNF-α standards were prepared in TNF-α free human serum and similarly supplemented with mouse serum and dithiothreitol. Each sample was assayed in duplicate on two separate microplates (n = 4). Four samples had a single outlier removed. The standard curves for both plates had R^2s of 0.99 over a concentration range of 0–250 pg/mL.

Statistical methods

A multilevel repeated measures analysis with auto-correlated time was conducted on the average COAT score and each of the four COAT subscales over the 85 measurement occasions from baseline (day 0) to completion (day 84) using SPSS (version 17.0; SPSS Inc, Chicago, IL, USA) with the Mixed procedure with a first order autoregressive (AR1) covariance modeled on the daily repeated measurements. The model presented fitted a two-piece linear response over time[29] with a change point at day 21, and included the effects of treatment, 2-piece time, and 2-piece time by treatment interaction.

Results

Study participants

The study screened volunteers and subsequently enrolled thirteen people who met the inclusion/exclusion criteria and were comprised of six females (mean age [±SD] 62.16 [±9.04] years) and seven males (mean age [±SD] 57.14 [±9.20] years). One subject withdrew a week prior to the start of the baseline measure due to an acute respiratory tract infection. Eleven participants completed 12 weeks of the study. One participant completed 10 weeks of the study due to overseas travel for the final 2 weeks and their final blood measurement was taken at week 10. Data was analysed for these 12 participants. Five participants received the formulation in doses of 100 mg Maritech® extract and seven received 1000 mg Maritech® extract. There was 99.2% compliance at four weeks and 99.6% at twelve weeks with participants taking 100 mg daily and 97.4% at four weeks and 95.9% compliance at twelve weeks in individuals taking 1000 mg per day. Please see Figure 1 for a flow diagram of the trial. The study medication was well tolerated as all 12 participants were satisfied with the effects of taking the

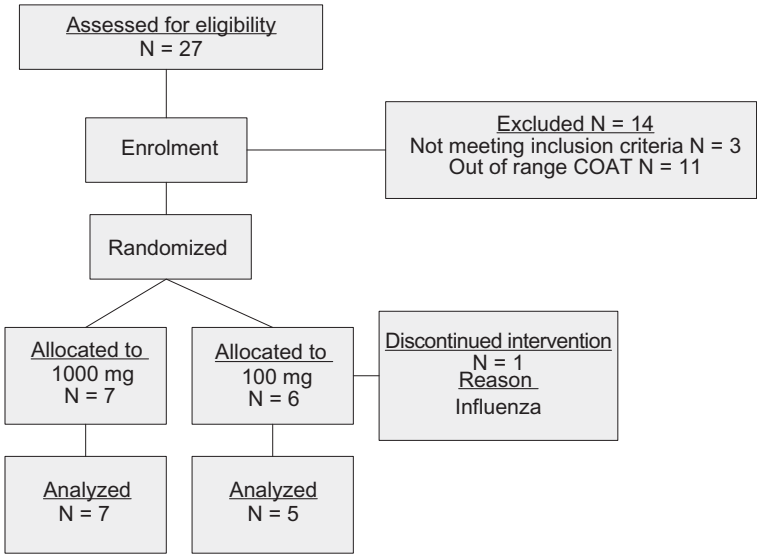

Figure 1 Flow diagram of trial organization.

study medication (compared with dissatisfied; or neither satisfied or dissatisfied).

The mean ages, and the baseline means for blood pressure, weight and pulse rate for the 100 mg and 1000 mg arms were not statistically different (data not presented).

COAT scores

Subjects taking either dose experienced reduction in COAT scores over the 12 weeks of the study. Means for the main outcome, the average of the COAT score subscales (average COAT), at baseline and on every seventh measurement occasion are provided for each treatment in Table 2 to summarize the response. A more complete description showing the daily mean average COAT scores is provided graphically in Figure 2 to which an empirical Loess curve was fitted to summarize the underlying response profiles. On observing Figure 2, two-piece linear functions with the break points at days 21 and 28 were tested in the statistical model for the Average COAT score, with the day 21 break point displaying superior fit in terms of the –2 log likelihood fit statistics (2007.9 at 21 days and 2010.7 at 28 days). Table 3 reports the parameter estimates for the average COAT model and Figure 3 shows the estimated mean average COAT profiles by time by treatment. There was a highly significant treatment by time interaction effect (likelihood ratio test, chi-square = 15.155, df = 2, $P = 0.0005$), which was largely located after day 21 as indicated by the tests of the interaction parameters in Table 3. The parameter estimates are not reported for the pain, stiffness, physical difficulties and overall symptoms sub-scales but were similar to those obtained for the average COAT scale as would be expected from highly correlated variables. Table 4 reports the estimated means for each of the COAT scales at baseline and completion for both treatments, and the results of a one-tailed test of the hypothesis that the reduction from baseline to completion was significantly greater in the 1000 mg than the 100 mg treatment. This hypothesis was confirmed at $P < 0.05$ for the average COAT (0.043) scale, and the physical difficulties (0.010) and overall symptoms (0.044) sub-scales, but failed to reach significance for the

Table 2 Average COAT score: mean, standard deviation (SD), minimum and maximum at the weekly measurement occasions by treatment

Week	100 mg					1000 mg				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Baseline	5	4.54	1.02	3.25	6.00	7	5.32	0.94	4.33	7.00
1	5	4.25	2.07	2.00	6.38	7	4.10	1.62	1.75	6.93
2	5	4.30	1.92	2.00	6.25	7	3.62	0.82	2.25	4.50
3	5	3.88	1.25	3.00	6.00	7	3.39	1.44	0.88	5.50
4	5	3.68	1.79	2.00	6.00	7	3.06	1.69	0.00	4.50
5	5	3.73	1.44	2.00	6.00	7	3.15	1.66	0.00	5.00
6	5	3.88	1.54	2.00	6.00	7	3.61	2.11	0.25	7.05
7	5	3.68	1.70	2.00	6.00	7	2.79	1.49	0.88	5.00
8	5	4.40	1.66	2.00	6.38	7	2.71	1.62	0.00	5.00
9	5	4.13	1.56	2.00	6.25	7	2.64	1.86	0.00	5.50
10	5	3.70	1.71	2.00	6.25	7	2.83	1.82	0.00	5.00
11	4	3.59	1.90	2.00	6.25	7	2.65	2.08	0.00	5.50
12	4	3.63	1.87	2.00	6.25	7	2.27	1.95	0.00	5.00

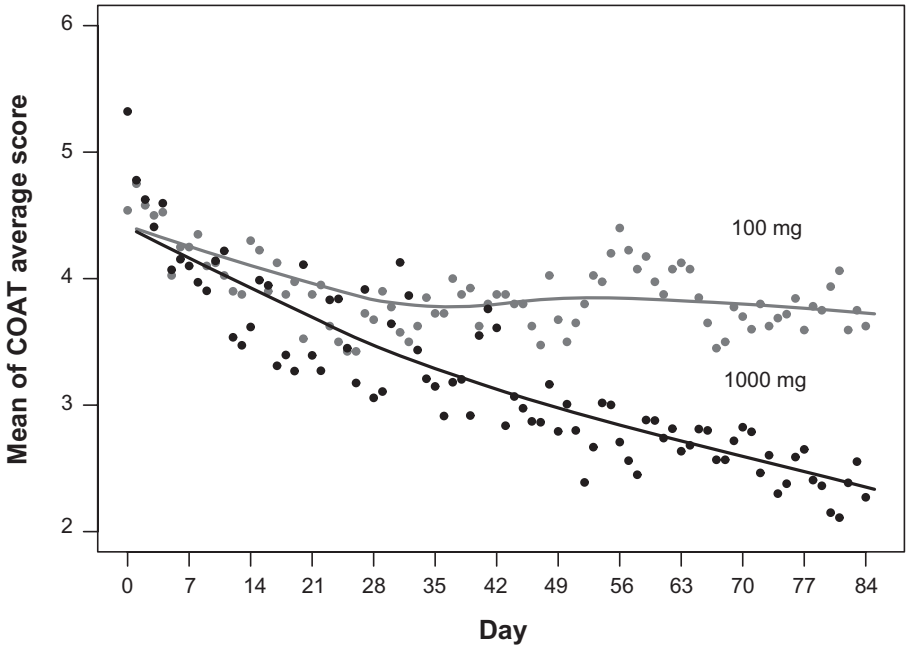

Figure 2 Average COAT score: daily mean by time by treatment with fitted Loess curves.

pain (0.088) and stiffness (0.089) subscales. As shown in Table 4, mean average COAT reduced from 4.54 to 3.72 (18%) in the 100 mg treatment and from 4.81 to 2.32 (52%) in the 1000 mg treatment.

Adverse events

Six adverse events were noted. The first event, influenza occurred prior to the commencement of the trial and the participant withdrew. Two participants had hypertension, one baseline and one at week 4. Both participants had a history of hypertension. One participant had a chest infection at week 12, one had root canal work at week 12, and one participant had hyperacidity at week 12 with a history of gastric acidity at baseline. All events were considered to be unlikely to be related to the study medication.

Blood safety measures

There were no toxicity issues observed over the period of the study hemopoietic, hepatic and renal systems. Due to the small numbers of participants in this study it is not possible to consider gender differences. Table 5 presents the mean, standard deviation (SD) and sample size for each blood safety measure at baseline and completion in each treatment and a test of the significance of the change. There were no statistically significant changes in either treatment over time although there was a statistically significant but not clinically

Table 3 Average COAT scores: parameter estimates with their standard errors and tests of significance

Parameter	Estimate	SE	t value	df	*P* value
Intercept (100 mg)	4.542	0.705	6.442	13.699	0.000
Treatment (1000 mg)	0.263	0.923	0.285	13.698	0.780
Time 0–21 (days)	–0.036	0.018	–2.014	108.787	0.047
Time 21–84	–0.001	0.005	–0.227	87.490	0.821
Time 0–21 by treatment (1000 mg)	–0.027	0.023	–1.166	108.725	0.246
Time 21–84 by treatment (1000 mg)	–0.017	0.007	–2.539	87.311	0.013
Random: Subject level variance	2.078	0.949	2.190		0.029
Daily variance AR1	0.739	0.059	12.528		0.000
rho AR1	0.680	0.025	26.749		0.000

Abbreviation: SE, standard error.

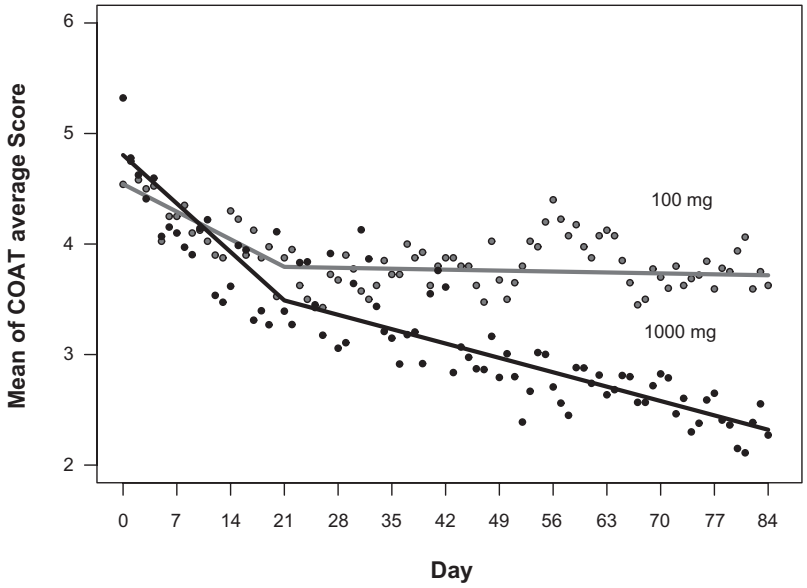

Figure 3 Average COAT score: estimated mean by time by treatment.

Table 4 COAT scales: estimated baseline and completion means with 95% confidence intervals (CI) and tests of the difference in the reduction from baseline to completion between treatments

COAT scale	Treatment	Time	Mean	Lower 95% CI	Upper 95% CI
Average	100 mg	Baseline	4.542	3.027	6.057
		Completion	3.717	2.236	5.199
	1000 mg	Baseline	4.805	3.524	6.086
		Completion	2.320	1.071	3.568
				Test[1]	$P = 0.043$
Pain	100 mg	Baseline	4.903	3.338	6.468
		Completion	3.827	2.300	5.355
	1000 mg	Baseline	4.786	3.464	6.109
		Completion	2.122	0.086	3.409
				Test[1]	$P = 0.088$
Stiffness	100 mg	Baseline	4.853	3.334	6.371
		Completion	3.605	2.123	5.088
	1000 mg	Baseline	4.720	3.437	6.004
		Completion	2.339	1.090	3.588
				Test[1]	$P = 0.089$
Physical difficulties	100 mg	Baseline	3.805	2.313	5.297
		Completion	3.667	2.209	5.125
	1000 mg	Baseline	4.803	3.543	6.064
		Completion	2.401	1.173	3.629
				Test[1]	$P = 0.010$
Overall	100 mg	Baseline	4.614	3.078	6.149
		Completion	3.769	2.267	5.271
	1000 mg	Baseline	4.868	3.571	6.166
		Completion	2.413	1.147	3.678
				Test[1]	$P = 0.044$

Note: [1]One-tailed test of the hypothesis that the reduction in mean scores from baseline to completion is greater in the 1000 mg than the 100 mg treatment.

Table 5 Blood safety measures: mean, standard deviation (SD) at baseline and completion 12 together with mean, SD, *P*-values for differences between baseline and completion by treatment

			Baseline		Week 12		Diff		
		N	Mean	SD	Mean	SD	Mean	SD	*P*
Hematology									
Red cell count ($x10^{12}$/L)	100 mg	5	4.88	0.35	4.82	0.25	0.06	0.17	0.468
	1000 mg	7	4.70	0.30	4.71	0.32	–0.01	0.22	0.869
Hemoglobin (g/L)	100 mg	5	150.40	8.35	147.20	4.60	3.20	5.85	0.288
	1000 mg	7	146.71	11.24	145.43	10.42	1.29	8.90	0.716
Hemacrit (%)	100 mg	5	0.44	0.03	0.44	0.02	0.00	0.02	0.799
	1000 mg	7	0.43	0.03	0.43	0.03	–0.01	0.03	0.578
Mean corpuscular volume (fL)	100 mg	5	90.20	2.68	78.00	27.50	12.20	26.19	0.356
	1000 mg	7	91.00	1.83	91.14	1.86	–0.14	1.68	0.829
White cell count ($x10^9$/L)	100 mg	5	5.76	0.98	5.84	1.30	–0.08	0.56	0.767
	1000 mg	7	6.59	1.19	6.00	0.79	0.59	1.42	0.318
Lymphocytes ($x10^9$/L)	100 mg	5	1.96	0.23	1.74	0.21	0.22	0.31	0.189
	1000 mg	7	2.04	0.32	1.96	0.29	0.09	0.38	0.573
Monocytes ($x10^9$/L)	100 mg	5	0.46	0.05	0.52	0.16	–0.06	0.15	0.426
	1000 mg	7	0.46	0.16	0.47	0.05	–0.01	0.15	0.805
Neutrophils ($x10^9$/L)	100 mg	5	3.08	0.89	3.30	0.88	–0.22	0.36	0.240
	1000 mg	7	3.89	0.77	3.37	0.65	0.51	0.93	0.192
Basophils ($x10^9$/L)	100 mg	5	0.02	0.04	0.02	0.04	0.00	0.00	
	1000 mg	7	0.00	0.00	0.00	0.00	0.00	0.00	
Eosinophils ($x10^9$/L)	100 mg	5	0.24	0.17	0.22	0.13	0.02	0.04	0.374
	1000 mg	7	0.17	0.05	0.17	0.05	0.00	0.06	10.000
Platelet count ($x10^9$/L)	100 mg	5	235.00	39.16	232.60	46.33	2.40	13.35	0.708
	1000 mg	7	277.71	61.35	265.57	46.18	12.14	28.64	0.305
Biochemistry									
Alkaline phosphatase (u/L)	100 mg	5	68.20	6.76	64.80	7.66	3.40	3.13	0.072
	1000 mg	7	71.71	8.44	68.14	12.95	3.57	11.72	0.451
Alanine aminotransferase (u/L)	100 mg	5	22.80	6.06	30.80	8.90	–8.00	9.19	0.124
	1000 mg	7	22.71	8.28	28.47	22.79	–5.76	15.64	0.368
Aspartate aminotransferase (u/L)	100 mg	5	25.20	11.28	30.00	10.12	–4.80	10.06	0.346
	1000 mg	7	21.14	4.41	23.86	5.70	–2.71	6.13	0.286
Y-glutamyl transferase (u/L)	100 mg	5	27.80	16.50	27.40	12.22	0.40	5.37	0.876
	1000 mg	7	33.29	17.07	39.71	35.99	–6.43	20.35	0.435
Bilirubin (µmol/L)	100 mg	5	12.60	4.16	11.20	2.39	1.40	3.78	0.454
	1000 mg	7	11.43	3.15	10.86	1.21	0.57	3.15	0.649
Protein (g/L)	100 mg	5	70.80	6.14	71.00	6.36	–0.20	2.49	0.866
	1000 mg	7	68.00	3.11	66.43	4.08	1.57	2.44	0.139
Albumin (g/L)	100 mg	5	44.40	2.88	46.00	1.87	–1.60	2.19	0.178
	1000 mg	7	43.00	1.00	43.57	1.13	–0.57	0.79	0.103
Sodium (mmol/L)	100 mg	5	138.80	0.45	139.80	0.84	–1.00	0.71	0.034
	1000 mg	7	138.86	1.35	138.57	1.27	0.29	1.50	0.631
Potassium (mmol/L)	100 mg	5	4.06	0.29	4.04	0.26	0.02	0.38	0.911
	1000 mg	7	4.17	0.21	3.97	0.29	0.20	0.43	0.263
Chloride (mmol/L)	100 mg	5	104.00	2.45	105.80	2.68	–1.80	1.10	0.021
	1000 mg	7	105.00	2.89	105.29	2.87	–0.29	2.43	0.766

(*Continued*)

Table 5 (*Continued*)

			Baseline		Week 12		Diff		
		N	Mean	SD	Mean	SD	Mean	SD	*P*
BUN/Urea (mmol/L)	100 mg	5	7.00	1.27	6.12	0.88	0.88	1.49	0.258
	1000 mg	7	5.77	1.08	6.34	0.82	−0.57	1.03	0.766
Creatine (mmol/L)	100 mg	5	98.00	9.57	89.60	7.64	8.40	3.91	0.009
	1000 mg	7	85.43	11.67	87.00	12.22	−1.57	11.57	0.732

Abbreviation: BUN, blood urea nitrogen.

significant increase in albumin over time for both treatments combined ($P = 0.034$).

Paracetamol use

Descriptive statistics for paracetamol use are reported in Table 6 together with Mann–Whitney tests comparing the treatments on use at baseline and total use over the duration of the trial. Weekly total paracetamol use (500 mg tablets) is plotted by treatment in Figure 4. None of subjects in the 100 mg treatment took any paracetamol on the first day of treatment while, of the seven subjects in the 1000 mg treatment, four took no paracetamol with the remaining three taking eight 500 mg tablets between them. The subjects in the 100 mg treatment took a total of 58 tablets (each 500 mg) over the duration of the trial with individual consumption ranging from 0 to 40 tablets, while the subjects in the 1000 mg treatment took a total of 152 tablets with individual consumption ranging from 0 to 62 tablets. Mann–Whitney comparisons of the two treatments showed no significant difference in consumption between the two groups either at baseline or over the duration of the trial.

TNF-α

Descriptive statistics for TNF-α are given in Table 7. There were no changes in TNF-alpha serum levels over time ($P = 0.170$), nor any differences between treatment groups ($P = 0.227$).

Cholesterol and serum lipids

Repeated measures analysis of variance was conducted on the baseline and 12 week values for serum lipids. Descriptive data is given in Table 8. One subject in the 100 mg group did not have a recordable low-density lipoprotein (LDL) in week 12. There were no differences found between the doses.

Discussion

This study investigated the effects on the symptoms of osteoarthritis using two doses of a complex containing a blend of Maritech® extracts from three brown algae species (*Fucus vesiculosis, Macrocystis pyrifera, Laminaria japonica.*) plus vitamin B6, zinc and manganese in an open label design. There was a clear dose dependent effect on symptoms of osteoarthritis as assessed using the COAT index. The preparation was found to be safe at the doses used in the study population over 12 weeks.

After 12 weeks, the 100 mg dose reduced the average COAT score by 18% and the 1000 mg dose by 52%. The decrease in OA symptoms demonstrated by the 1000 mg dose is marked. Whilst there is no direct comparator in this study, results expected from nonsteroid anti-inflammatory drugs (NSAIDS) produce similar reductions in OA symptoms.[30] Osteoarthritis studies often have large placebo responses and a limitation of this early investigation was a lack of a placebo arm. The significant difference between the two doses in a dose-dependent progression increases the likelihood that the 1000 mg dose is superior to a placebo.

A recent randomised controlled study on a mineral based supplement derived from seaweed undertaken over 12 weeks demonstrated changes in the symptoms of osteoarthritis when measured by WOMAC.[8] Although derived from a seaweed, the mineral-based supplement was substantially different

Table 6 Paracetamol usage: mean, standard deviation (SD), and mean rank by treatment at baseline and over the duration of the trial with the Mann–Whitney *P*-values

	100 mg				1000 mg				Mann-Whitney
	N	Mean	SD	Mean rank	N	Mean	SD	Mean rank	*P*
Baseline (Day 1)	5	0.00	0.00	5.00	7	1.14	1.57	7.57	0.205
Full trial (Day 1 to Day 84)	5	11.60	16.15	5.80	7	21.71	25.65	7.00	0.615

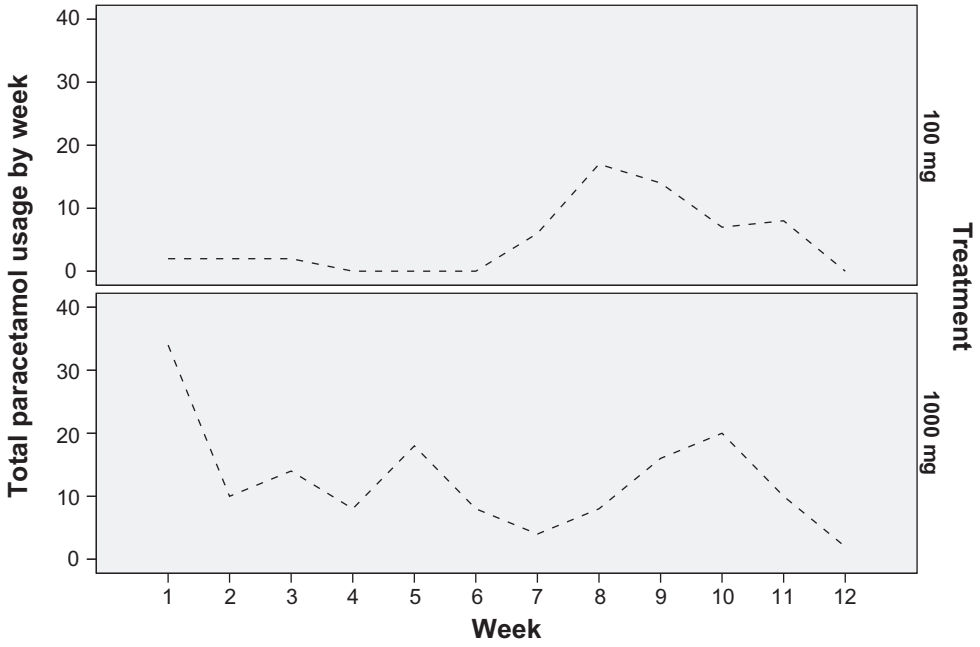

Figure 4 Paracetamol usage: weekly total tablets (500 mg) by treatment.

from the soluble fucoidan-rich preparations used in this study, and cannot be directly compared.

Individuals with OA show raised levels of TNF-α,[31] and we hypothesized that the seaweed formulation would demonstrate a dose-dependent reduction over time as the fucoidans and polyphenols have been identified as anti-inflammatory agents. In this study, the TNF-α levels showed no change at either dosage perhaps indicating that the effect observed is not mediated via a TNF-α-associated cascade. However the study is not sufficiently powered to rule out a reduction in TNF-α, and further studies should include the measure.

Fucoidan is a major component of Maritech® seaweed extracts. It is not a precursor to mammalian tissue formation, and is generally assumed to be impervious to mammalian enzyme breakdown. Fucoidans have profound selectin-blocking activity,[18] and serum uptake of fucoidan has been demonstrated previously.[17] It is possible that, in this clinical trial, selectin-blocking effects may reduce leukocyte accumulation in the osteoarthritis affected areas, reducing the COAT scores via a generalized inhibition of inflammation. However paradoxically others have argued that selectin blockade may actually reduce pain relief.[32] Further clinical trials and laboratory investigations are necessary to elucidate its mechanism of action.

Table 7 Tumor necrosis factor alpha levels: means, standard deviation (SD), minima and maxima by treatment arm and measurement occasion

Occasion	100 mg					1000 mg				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Baseline	5	10.28	2.18	7.89	13.12	7	9.54	1.53	6.97	11.84
Week 4	5	9.08	2.24	7.20	12.74	7	9.66	1.43	6.93	11.44
Week 12	5	10.85	2.06	8.66	13.35	7	9.80	1.50	7.51	11.70

The preparation was shown to be safe at the dosages consumed by the study population and any adverse effects were mild and self limiting. There were no changes in the cholesterol, liver function, renal function, and hemopoietic function that were of any clinical significance during the course of the study.

The most significant limitation of this study was; being a pilot scale open label combined phase I and II trial it was subject to potential bias that would be reduced by the use of randomization to placebo or active with appropriate blinding. This study aimed to determine if this preparation had any potential in the treatment of the symptoms of osteoarthritis. The study achieved this aim and the significant dose responsiveness demonstrated provides evidence that these results are unlikely to be due to chance and deserve further exploration.

Conclusion

A seaweed extract nutrient complex when taken orally over twelve weeks significantly reduced the symptoms of osteoarthritis in a phase I and II open label study. The effect was highly dose-dependent with final reduction in COAT score of

Table 8 Cholesterol, HDL, LDL, ratio and triglycerides: means, SD, minima and maxima by treatment and measurement

Cholesterol (mmol/L)	100 mg					1000 mg				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
Baseline	5	5.60	1.28	4.50	7.80	7	5.19	0.65	4.40	6.40
HDL	5	1.21	0.35	0.73	1.61	7	1.29	0.22	1.10	1.72
LDL	5	3.44	0.77	2.70	4.70	7	3.23	0.64	2.30	4.30
Ratio	5	5.30	1.32	4.00	7.40	7	4.13	0.93	2.70	5.30
Triglycerides	5	2.10	1.40	0.70	3.80	7	1.43	0.48	0.60	1.90
Week 12	5	5.44	1.52	3.70	7.90	7	5.17	0.58	4.20	6.10
HDL	5	1.18	0.34	0.79	1.59	7	1.39	0.24	1.11	1.78
LDL	4	2.93	0.43	2.30	3.30	7	3.19	0.61	2.00	4.00
Ratio	5	4.52	2.23	1.59	6.80	7	3.60	1.25	1.59	4.90
Triglycerides	5	2.56	2.46	1.00	6.80	7	1.27	0.61	0.60	2.40

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

18% and 52% respectively for the 100 mg and 1000 mg doses. The preparation was demonstrated to be safe to use over the study period at the doses tested in the study population. The observed pharmacological benefits now need to be demonstrated in a phase III randomized controlled trial.

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