SEA BUCKTHORN FRUIT OIL SUPPLEMENTATION IN ACTIVE RHEUMATOID ARTHRITIS: A FOCUS ON COMPOSITE DISEASE ACTIVITY INDICES AND EULAR RESPONSE

Georgeta Stefanovici Zugravu[1,5], Carmen Pintilescu[6], Carmen Marinela Cumpat[2,5], S. D. Miron[3], Doina-Clementina Cojocaru[2,5], Anca Miron[1]
"Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania
Faculty of Pharmacy
1. Department of Pharmaceutical Sciences (II)
Faculty of Medicine
2. Department of Medical Specialties (III)
3. Department of Morpho-Functional Sciences (II)
4. Department of Medical Specialties (I)
5. Clinical Rehabilitation Hospital, Iasi, Romania
"Alexandru Ioan Cuza" University of Iasi, Romania
Faculty of Economics and Business Administration
6. Department of Accounting, Business Information Systems and Statistics
*Corresponding author. E-mail: anca.miron@umfiasi.ro

SEA BUCKTHORN FRUIT OIL SUPPLEMENTATION IN ACTIVE RHEUMATOID ARTHRITIS: A FOCUS ON COMPOSITE DISEASE ACTIVITY INDICES AND EULAR RESPONSE (Abstract): Rheumatoid arthritis is a disabling disease whose treatment options have limitations with regard to efficacy and safety. In this respect, combination of conventional treatment with plant-derived products has attracted considerable interest in recent years. The aim of this study was to assess the impact of sea buckthorn fruit oil supplementation in patients with active rheumatoid arthritis. **Materials and methods:** Eighty patients, diagnosed with active rheumatoid arthritis and treated with a combination of methotrexate (15 mg/week) and diclofenac sodium (50 mg/day), were randomly (1:1) divided into control and experimental groups, the latter being supplemented with sea buckthorn fruit oil (1800 mg/day). The study lasted 12 weeks. The benefits of sea buckthorn fruit oil supplementation were evaluated by various composite disease activity indices (disease activity score in 28 joints, DAS28; simplified disease activity index, SDAI; clinical disease activity index, CDAI) and European League Against Rheumatism (EULAR) response. **Results:** Twelve-week supplementation with sea buckthorn fruit oil significantly ($p< 0.001$) reduced composite disease activity indices in comparison with baseline and control. At week 12, patients in the experimental group showed higher rates of good and moderate EULAR responses than those in the control group. **Conclusions:** According to our results, sea buckthorn fruit oil is a promising option for the adjunctive treatment in rheumatoid arthritis. **Keywords:** SEA BUCKTHORN FRUIT OIL, ACTIVE RHEUMATOID ARTHRITIS, COMPOSITE DISEASE ACTIVITY INDEX, EULAR RESPONSE.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune-inflammatory disease characterized by articular and extraarticular manifestations (synovial membrane inflammation, articular cartilage destruc-

tion, periarticular bone erosion and subcutaneous nodules, lung disease, pericarditis, respectively). If inadequately treated, it results in permanent joint deformities, functional disability and poor life quality (1, 2). The drugs used to treat RA, specifically corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), conventional and biological disease-modifying antirheumatic drugs (DMARDs), produce severe side effects. In addition, none of these drugs, alone or in combination, induce a medication-free remission. For this reason, supplementation with plant-derived products to support the efficacy and safety of conventional treatment has attracted considerable interest in recent years (1).

The benefits of coadministration of herbal extracts/phytochemicals with antiarthritic drugs were demonstrated in clinical studies. In RA patients, *Tripterygium wilfordii* Hook.f. extracts improved the therapeutic efficacy of methotrexate (MTX) as evaluated by clinical outcomes and reduction in inflammatory and diagnostic markers (erythrocyte sedimentation rate (ESR), C reactive protein (CRP) and rheumatoid factor (RF), respectively). Supplementation with total glucosides of peony (*Paeonia lactiflora* Pall.) in RA patients, treated with MTX or MTX in combination with leflunomide, reduced liver toxicity caused by DMARDs, ameliorated clinical symptoms, ESR, CRP, RF and lipid profile (3). The combination of dry olive (*Olea europaea* L.) leaf extract with MTX was more efficient than MTX alone in restoring antioxidant defense and reducing oxidative stress, cell damage and interleukin-6 (IL-6) production in early phase RA patients (4). Supplementation with garlic (*Allium sativum* L.) powder in patients with moderate to severe RA, under treatment with DMARDs, resulted in improved clinical symptoms and significant reductions in CRP and tumor necrosis factor- α (TNF-α) compared to patients receiving only DMARDs (5). Also, combinations of pure phytochemicals with antiarthritic drugs were reported to exert better activity and less toxicity than antiarthritic drugs alone. Combined administration of resveratrol with conventional DMARDs induced greater improvement in clinical, biochemical (RF, ESR, CRP, TNF-α, IL-6) and hip fracture (undercarboxylated osteocalcin) markers (6). Sesamin supplementation attenuated pain intensity and significantly decreased CRP, TNF-α, cyclooxygenase-2 (COX-2), hyaluronidase and matrix metalloproteinase-3 (MMP-3)(7). In RA patients, the therapeutic efficacy of sinomenine in combination with MTX was comparable with that of MTX-leflunomide combination but the safety and tolerability (with respect to gastrointestinal and hepatic side effects) were better (8). The combination of curcumin with diclofenac sodium (DicNa) induced better clinical outcomes in comparison with DicNa in patients with active RA (9).

Due to a unique mixture of bioactive compounds, sea buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson syn. *Hippophae rhamnoides* L., Elaeagnaceae) fruit oil is one of the most valuable plant-derived products. The oil obtained from whole berries is rich in palmitoleic acid (11-37%), an ω-7 fatty acid, other unsaturated fatty acids such as ω-3, ω-6 and ω-9 fatty acids (3-8% α-linolenic acid, 12-18% linoleic acid and 20-53% oleic acid, respectively) and saturated fatty acids (23-40% palmitic acid, < 2% stearic acid), sterols (1200-2300 mg/100 g), carotenoids (350-520 mg/100 g) and tocopherols (up to 160 mg%, α-tocopherol being predominant) (10, 11). Sea buckthorn fruit oil has applications in skin and mucosal disorders (wounds, burns, lesions caused by radiation exposure, hyper pigmentation, infections, itchiness, vaginal

inflammatory atrophy). As nutraceutical, sea buckthorn fruit oil has benefits in the prevention and adjunctive treatment of cardiovascular, liver and metabolic diseases. In cancer patients undergoing chemotherapy and radiotherapy, sea buckthorn fruit oil counteracts some of treatment side effects such as alterations in liver and kidney function and appetite loss (10-12).

The current study was designed to evaluate the efficacy and safety of sea buckthorn fruit oil (SBFO) supplementation in patients with active RA. Disease activity score in 28 joints (DAS28), simplified disease activity index (SDAI), clinical disease activity index (CDAI)and European League Against Rheumatism (EULAR)response were used as efficacy indicators.

MATERIALS AND METHODS

A pilot randomized controlled study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Institutional Ethics Committee of *Grigore T. Popa* University of Medicine and Pharmacy Iasi, Romania (approval no. 68/13.04.2021) and Ethics Committee of the Clinical Rehabilitation Hospital Iasi, Romania (approval no. 13/20.04.2021).All enrolled patients filled in and signed the written informed consent. In brief, 80 patients diagnosed with active RA were randomly classified (1:1) into two groups: control group, treated with MTX (15 mg/week) and DicNa (50 mg/day) and experimental group, receiving the aforementioned medication and SBFO (1800 mg/day, Sea Buckthorn Oil 900 mg, S.C. Hofigal Export Import S.A., Bucharest, Romania). The study lasted 12 weeks. The inclusion and exclusion criteria and baseline characteristics of patients were described in detail elsewhere (Zugravu *et al.*, submitted).

The inflammatory markers (ESR, CRP),lipid profile, glycemia, liver and renal function markers were assessed at baseline and week 12. Venous blood (10 mL) was collected after fasting (10-12 h). A volume of 2 mL was used for ESR measurement (Westergren method, THERMA analyzer, Linear Chemicals, Montgat-Barcelona, Spain). The remaining volume was centrifuged (3000 g, 10 min.) to separate serum and plasma which were further stored at -80°C. Serum CRP was determined by immunoturbidimetry (biochemical analyzer XL 1000, Erba, Lachema S.R.O., Czech Republic). Liver and kidney function markers, lipid and carbohydrate metabolic markers were determined spectrophotometrically (biochemical analyzer XL 1000, Erba, Lachema S.R.O., Czech Republic).

The clinical variables, assessed at baseline and week 12, were the tender joint count (in 28 and 68 joints, TJC28 and TJC68), swollen joint count (in 28 and 66 joints, SJC28 and SJC66) and morning stiffness (in min.) (13, 14). Composite disease activity indices such as DAS28 (15), SDAI andCDAI (13) were also evaluated at the beginning and end of the study. EULAR responses were assessed at week 12 according to improve mentin DAS28 score (15). DAS28, SDAI, CDAIand EULAR responses were calculated as previously reported (13, 15).

Data were analyzed using the SPSS software (version 25). The Kolmogorov-Smirnov test was initially used to assess the normality of data distribution. The differences between the two groups (experimental and control groups) at each evaluation (baseline and week 12) and the differences between the evaluations for each group were examined using either parametric or nonparametric tests. The independent samples *t*-test and Mann-Whitney test were used to compare the differences between experimental and control groups. Within

each group, the comparisons were done using the paired samples *t*-test (normally distributed data) and Wilcoxon signed-rank test (non-normally distributed data). The Chi-square test was applied to assess the relationship between two qualitative variables and two patient groups. Values of *p* less than or equal to 0.05 were considered statistically significant.

RESULTS

The inflammatory markers (ESR, CRP) were not significantly ($p > 0.05$)different between the two patient groups at baseline and week 12. At week 12, CRP decreased in both experimental and control groups; ESR had slightly reduced values in the control group and unexpectedly higher values in the experimental group (tab. I).

TABLE I.
Inflammatory markers at baseline and week 12

Inflammatory marker	Control group	Experimental group	*p* value
ESR (mm/h)			
Baseline	29.57 ± 22.74	27.12 ± 20.59	0.615[a]
(95% CI)	(22.30 – 36.84)	(20.53 – 33.71)	
Week 12	28.38 ± 23.61	32.10 ± 26.46	0.519[a]
(95% CI)	(20.73 – 36.03)	(23.28 – 40.93)	
Change	1.02 ± 12.43	-4.21 ± 20.96	-
p value	0.609[b]	0.229[b]	-
CRP (mg/dL)			
Baseline	17.50 ± 29.95	9.44 ± 19.37	0.158[a]
(95% CI)	(7.92 – 27.08)	(3.25 – 15.64)	
Week 12	14.08 ± 34.44	7.51 ± 9.51	0.258[a]
(95% CI)	(2.92 – 25.25)	(4.34 – 10.68)	
Change	3.81 ± 17.32	2.43 ± 14.18	-
p value	0.177[b]	0.303[b]	-

Values are expressed as mean ± standard deviation for normally distributed variables and median (25th and 75th percentiles) for non-normally distributed variables and 95% confidence interval; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; [a] Independent t test; [b] Paired t test.

At baseline, there were no significant ($p > 0.05$) differences between the two groups with respect to the clinical variables (TJC28, TJC68, SJC28, SJC66, morning stiffness) and composite indices of disease activity such as DAS28 calculated with ESR or CRP (DAS28 (ESR) and DAS28 (CRP), respectively), SDAI and CDAI. In the experimental group, supplementation with SBFO for 12 weeks markedly ($p< 0.001$) improved all clinical variables and indices with significant differences between the values at baseline and week 12. At week 12, all clinical variables were significantly ($p< 0.001$) different between the experimental and control groups. On the other hand, at the end of the study, in the control group, the scores of clinical variables (except TJC28 and morning stiffness) significantly ($p \leq 0.001$) increased indicating higher disease activity and deterioration of patient condition (tab. II).

TABLE II.
Clinical variables at baseline and week 12

Outcome	Control group	Experimental group	*p* value
TJC28			
Baseline	19.72 ± 3.88	18.65 ± 4.58	0.261[a]
(95% CI)	(18.48 – 20.96)	(17.18 – 20.11)	
Week 12	20.43 ± 3.44	12.21 ± 2.83	< 0.001[a]
(95% CI)	(19.31 – 21.55)	(11.26 – 13.16)	
Change	-0.82 ± 3.03	6.94 ± 3.09	-
p value	0.099[b]	< 0.001[b]	-
TJC68			
Baseline	35.65 ± 10.05	32.90 ± 9.68	0.217[a]
(95% CI)	(32.43 – 38.86)	(29.80 – 35.99)	
Week 12	38.48 ± 9.01	19.54 ± 4.54	< 0.001[a]
(95% CI)	(35.56 – 41.40)	(18.02 – 21.05)	
Change	-3.10 ± 5.53	14.37 ± 8.69	-
p value	0.001[b]	< 0.001[b]	-
SJC28			
Baseline	10.62 ± 2.68	9.45 ± 3.10	0.074[a]
(95% CI)	(9.76 – 11.48)	(8.45 – 10.44)	
Week 12	13.05 ± 2.41	5.81 ± 2.39	< 0.001[a]
(95% CI)	(12.26 – 13.83)	(5.01 – 6.61)	
Change	-2.35 ± 2.13	3.89 ± 3.30	-
p value	< 0.001[b]	< 0.001[b]	-
SJC66			
Baseline	15.25 ± 4.35	14.42 ± 4.51	0.408[a]
(95% CI)	(13.85 – 16.64)	(12.98 – 15.86)	
Week 12	20.46 ± 5.08	8.91 ± 3.92	< 0.001[a]
(95% CI)	(18.81 – 22.11)	(7.61 – 10.22)	
Change	-2.35± 3.78	3.89 ± 5.50	-
p value	< 0.001[b]	< 0.001[b]	-
Morning stiffness (min.)			
Baseline	40.12 ± 22.68	40.12 ± 18.30	1.000[a]
(95% CI)	(32.86 – 47.38)	(34.26 – 45.98)	
Week 12	45.12 ± 16.28	21.62 ± 7.64	< 0.001[a]
(95% CI)	(39.85 – 50.41)	(19.07 – 24.16)	
Change	-4.74 ± 16.73	19.45 ± 18.17	-
p value	0.084[b]	< 0.001[b]	-

Values are expressed as mean ± standard deviation for normally distributed variables and median (25th and 75th percentiles) for non-normally distributed variables and 95% confidence interval (CI); TJC28, tender joint count in 28 joints; TJC68, tender joint count in 68 joints; SJC28, swollen joint count in 28 joints; SJC66, swollen joint count in 66 joints; a Independent t test; b Paired t test.

The composite indicesDAS28 (ESR), DAS28 (CRP), SDAI and CDAI were used to assess disease activity. DAS28 > 5.1, 3.2 < DAS28 ≤ 5.1 and DAS28 ≤ 3.2 indicate high, moderate and low disease activity, respectively; DAS28 less than 2.6 is an indicative of remission. SDAI > 26, 11 < SDAI ≤ 26 and 3.3 < SDAI ≤ 11 define high, moderate and low activity, respectively whereas SDAI ≤ 3.3 indicates remission. CDAI > 22, 10 < CDAI ≤ 22, 2.8 < CDAI ≤ 10 and CDAI ≤ 2.8 correspond to high, moderate, low activity and remission, respectively (13). According to the mean values of these composite indices, patients in both groups had high disease activity at baseline (DAS28 (ESR)/(CRP) > 5.1, SDAI > 26, CDAI > 22). At week 12, in the control group, patients still had high disease activity whereas in the experimental group, patients achieved moderate disease activity according to DAS28 (CRP) and SDAI (4.66 ± 0.58 and 25.13 ± 6.70, respectively). However, it is worthy to note that supplementation with SBFO induced significant (p< 0.001) reductions in DAS28 (ESR)/(CRP), SDAI and CDAI compared to baseline and control (tab.III). As shown in fig. 1-3, at baseline, 87.2-97.4% patients in the control group and 89.2-100% patients in the experimental group had high disease activity according to DAS28 (ESR)/DAS28 (CRP)/SDAI/CDAI scores. At week 12, the percentage of patients with high disease activity increased in the control group (from 94.9 to 100% according to DAS28 (ESR), from 87.2 to 97.4% according to DAS28 (CRP), from 97.4 to 100% according to SDAI and CDAI). In the experimental group, 12-week supplementation with SBFO resulted in a marked decline in patients presenting high disease activity (from 97.3 to 64.9% according to DAS28 (ESR), from 89.2 to 13.5% according to DAS28 (CRP), from 94.6 to 48.6% according to SDAI, from 100 to 64.9% according to CDAI) with a concomitant elevation in patients with moderate disease activity (from 2.7 to 35.1% according to DAS28 (ESR), from 10.8 to 86.5% according to DAS28 (CRP), from 5.4 to 51.4% according to SDAI, from 0 to 35.1% according to CDAI).

After 12-week supplementation, SBFO significantly (p< 0.001) improved EULAR-responses calculated with ESR or CRP (EULAR (ESR) and EULAR (CRP), respectively).Thus, in the experimental group, patients attained good and moderate EULAR responses in high rates (EULAR (ESR) good response: 35.1% *vs.* 0% in the control group, EULAR (CRP) good response: 59.5% *vs.* 0% in the control group, EULAR (ESR) moderate response: 56.8% *vs.* 2.6% in the control group, EULAR (CRP) moderate response: 40.5% *vs.* 0% in the control group) (fig.4). Most of the clinical laboratory parameters were not significantly different between the experimental and control groups at the beginning and end of the study. After 12-week supplementation, SBFO reduced total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and increased triglycerides (TG) but the differences compared to baseline and control were not significant (p > 0.05). Surprisingly, SBFO caused a significant (p< 0.001) reduction in high-density lipoprotein cholesterol (HDL-C). SBFO improved liver function as evaluated by aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) levels. At week 12, in the experimental group, all three enzymes showed lower levels compared to baseline (p > 0.05) whereas higher levels compared to baseline were detected in the control group (p > 0.05). SBFO supplemen-

tation significantly ($p< 0.05$) decreased ALT compared to control. SBFO supplementation also reduced serum levels of urea and uric acid and had little impact on creatinine ($p > 0.05$ compared to baseline). At week 12, urea and creatinine levels were significantly ($p = 0.001$) lower in the experimental group compared to control. A nonsignificant ($p > 0.05$) increase in glycemia and serum alkaline phosphatase (ALP), marker of liver damage, was detected in the experimental group at week 12 (tab. IV). A single adverse event was reported during the study period: one patient in the control group suffered from nausea after MTX administration.

TABLE III.
Composite indices at baseline and week 12

Outcome	Control group	Experimental group	*p* value
DAS28 (ESR)			
Baseline	6.50 ± 0.71	6.34 ± 0.69	0.292[a]
(95% CI)	(6.27 – 6.73)	(6.11 – 6.56)	
Week 12	6.68 ± 0.71	5.31 ± 0.64	< 0.001[a]
(95% CI)	(6.45 – 6.91)	(5.10 – 5.53)	
Change	-0.18 ± 0.46	1.09 ± 0.40	-
p value	0.017[b]	< 0.001[b]	-
DAS28 (CRP)			
Baseline	6.06 ± 0.59	5.81 ± 0.66	0.076[a]
(95% CI)	(5.87 – 6.25)	(5.60 – 6.02)	
Week 12	6.21 ± 0.58	4.66 ± 0.58	< 0.001[a]
(95% CI)	(6.02 – 6.40)	(4.46 – 4.85)	
Change	-0.13 ± 0.37	1.23 ± 0.40	-
p value	0.030[b]	< 0.001[b]	-
SDAI			
Baseline	43.94 ± 7.98	42.30 ± 9.39	0.401[a]
(95% CI)	(41.39 – 46.49)	(39.29 – 45.30)	
Week 12	49.56 ± 8.58	25.13 ± 6.70	< 0.001[a]
(95% CI)	(46.78 – 52.35)	(22.89 – 27.36)	
Change	-5.58 ± 9.72	18.19 ± 4.88	-
p value	0.001[b]	< 0.001[b]	-
CDAI			
Baseline	42.35 ± 9.06	41.35 ± 8.88	0.620[a]
(95% CI)	(39.45 – 45.24)	(38.50 – 44.19)	
Week 12	48.17 ± 7.23	24.35 ± 6.25	< 0.001[a]
(95% CI)	(45.83 – 50.52)	(22.26 – 26.43)	
Change	-5.82 ± 8.73	17.97 ± 5.35	-
p value	< 0.001[b]	< 0.001[b]	-

Values are expressed as mean ± standard deviation for normally distributed variables and median (25^{th} and 75^{th} percentiles) for non-normally distributed variables and 95% confidence interval (CI); DAS28 (ESR), disease activity score in 28 joints calculated with erythrocyte sedimentation rate; DAS28 (CRP), disease activity score in 28 joints calculated with C reactive protein; SDAI, simplified disease activity index; CDAI, clinical disease activity index; a Independent t test; b Paired t test.

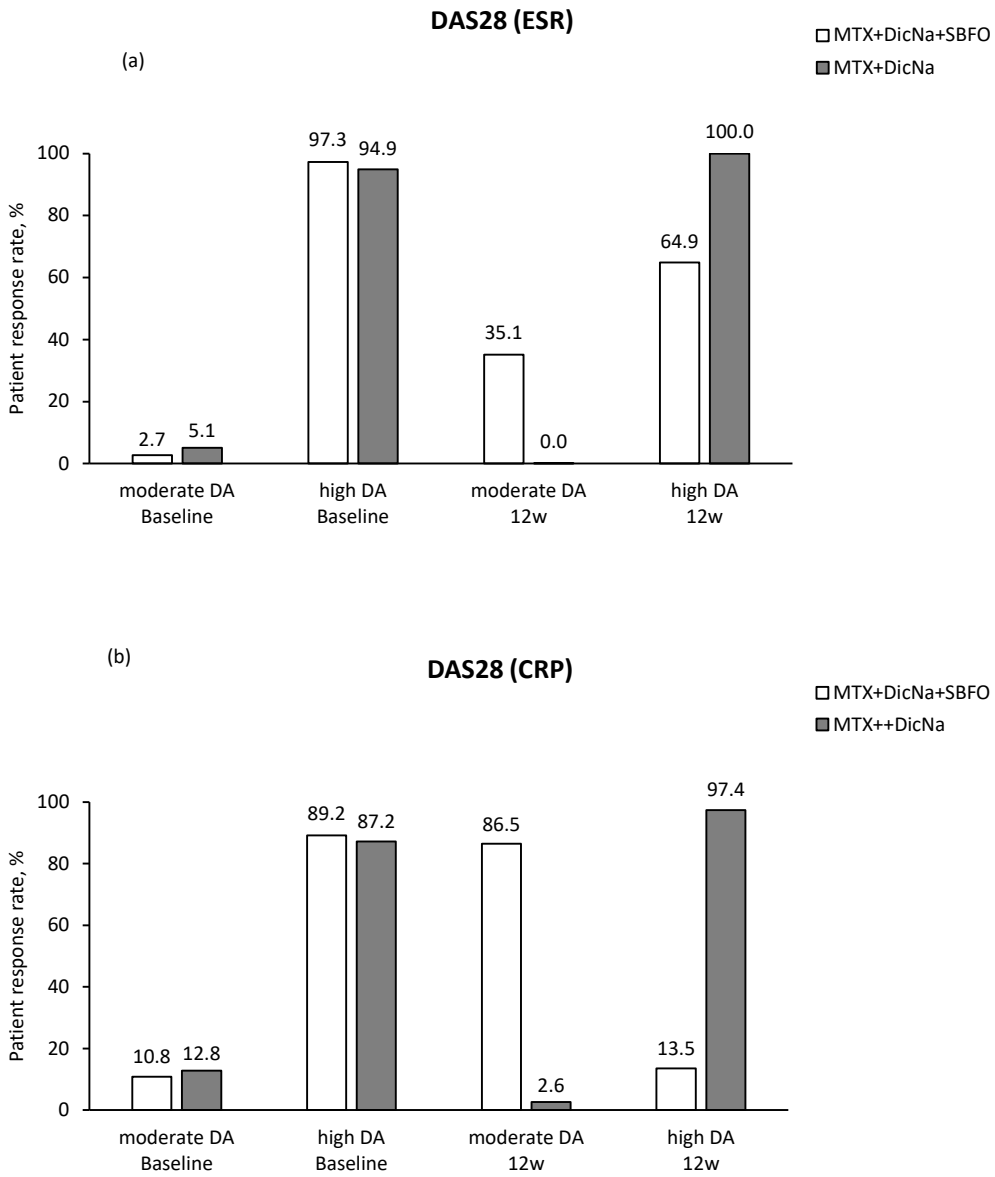

Fig. 1. Differences in DAS28 (ESR) (a) and DAS28 (CRP) (b) between the experimental and control groups (DAS28 (ESR), disease activity score in 28 joints calculated with erythrocyte sedimentation rate; DAS28 (CRP), disease activity score in 28 joints calculated with C reactive protein; DA, disease activity; MTX, methotrexate; DicNa, diclofenac sodium; SBFO, sea buckthorn fruit oil)

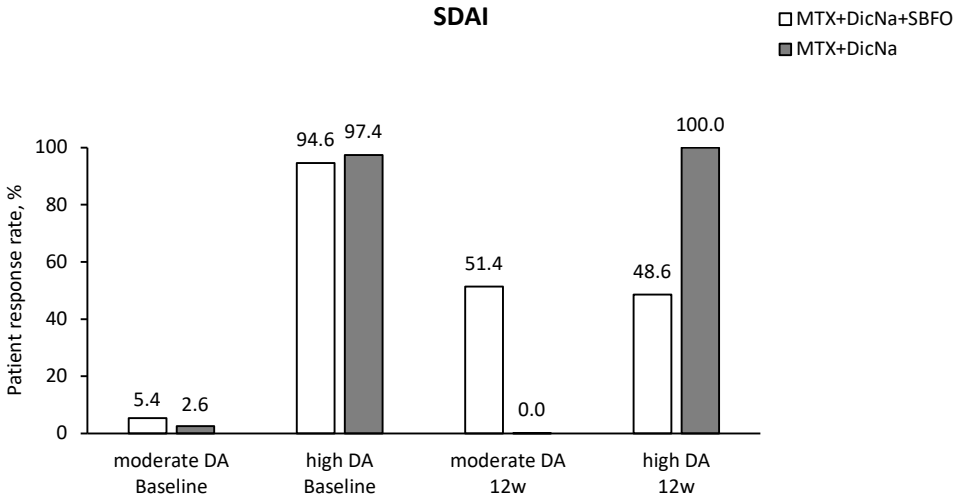

Fig. 2. Differences in SDAI between the experimental and control groups (SDAI, simplified disease activity index; DA, disease activity; MTX, methotrexate; DicNa, diclofenac sodium; SBFO, sea buckthorn fruit oil)

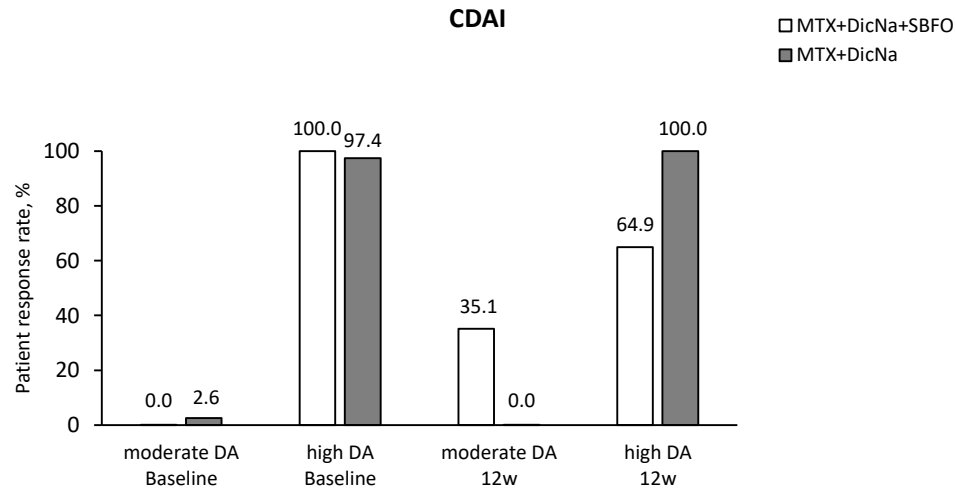

Fig. 3. Differences inCDAI between the experimental and control groups (CDAI, clinical disease activity index; DA, disease activity; MTX, methotrexate; DicNa, diclofenac sodium; SBFO, sea buckthorn fruit oil)

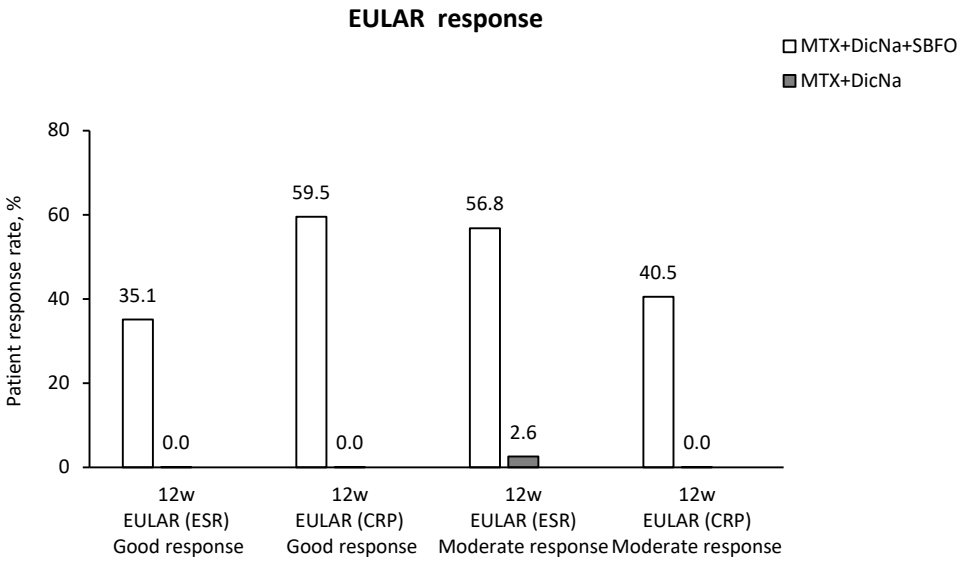

Fig. 4. Differences in EULAR (ESR)/(CRP) responses between the experimental and control groups (EULAR (ESR)/(CRP), European League Against Rheumatism response based on erythrocyte sedimentation rate/C reactive protein; MTX, methotrexate; DicNa, diclofenac sodium; SBFO, sea buckthorn fruit oil)

TABLE IV.

Clinical laboratory parameters at baseline and week 12

Variable	Control group	Experimental group	*p* value
TC (mg/dL)			
Baseline	213.7 ± 44.4	216.30 ± 47.82	0.646[a]
(95% CI)	(199.5 – 227.9)	(201.0 – 231.5)	
Week 12	215.7 ± 44.5	211.30 ± 50.27	0.688[a]
(95% CI)	(201.2 – 230.1)	(194.5 – 228.0)	
Change	-2.92 ± 42.18	6.10 ± 38.23	-
p value	0.668[b]	0.338 [b]	-
HDL-C (mg/dL)			
Baseline	67.16 ± 18.07	72.95 ± 14.84	0.122[a]
(95% CI)	(61.38 – 72.94)	(68.20 – 77.70)	
Week 12	69.66 ± 17.57	65.04 ± 13.40	0.203[a]
(95% CI)	(63.97 – 75.36)	(60.57 – 69.51)	
Change	-2.53 ± 16.29	7.16 ± 12.02	-
p value	0.337[b]	< 0.001[b]	-

Variable	Control group	Experimental group	*p* value
LDL-C (mg/dL)			
Baseline	140.10 ± 35.35	140.20 ± 44.57	0.991[a]
(95% CI)	(128.80 – 151.4)	(126.0 – 154.5)	
Week 12	134.20 ± 35.12	131.80 ± 40.03	0.781[a]
(95% CI)	(122.8 – 145.5)	(118.4 – 145.5)	
Change	5.11 ± 30.78	9.89 ± 34.75	-
p value	0.306[b]	0.092[b]	-
TG (mg/dL)			
Baseline	102.30 ± 46.91	96.36 ± 51.97	0.592[a]
(95% CI)	(87.32 – 117.30)	(79.74 – 113.00)	
Week 12	114.90 ± 52.14	108.30 ± 48.64	0.574[a]
(95% CI)	(97.99 – 131.8)	(92.12 – 124.6)	
Change	-12.92 ± 40.35	-9.35 ± 32.73	-
p value	0.053[b]	0.091[b]	-
GL (mg/dL)			
Baseline	112.50 ± 32.21	103.70 ± 29.81	0.209[a]
(95% CI)	(102.2 – 122.8)	(94.13 – 113.20)	
Week 12	108.50 ± 23.32	106.60 ± 23.13	0.712[a]
(95% CI)	(101.0 – 116.1)	(98.83 – 114.30)	
Change	4.1 ± 27.7	-7.09 ± 26.39	-
p value	0.362[b]	0.111 [b]	-
Urea (mg/dL)			
Baseline	39.70 ± 7.97	33.92 ± 10.74	0.009[a]
(95% CI)	(37.11 – 42.28)	(30.33 – 37.49)	
Week 12	37.04 ± 10.31	31.70 ± 6.71	0.001[a]
(95% CI)	(33.70 – 40.38)	(29.46 – 33.94)	
Change	2.66 ± 6.94	2.21 ± 9.11	-
p value	0.022[b]	0.148[b]	-
Creatinine (mg/dL)			
Baseline	1.03 ± 0.14	0.94 ± 0.11	0.002[a]
(95% CI)	(0.98 – 1.08)	(0.90 – 0.97)	
Week 12	1.04 ± 0.14	0.94 ± 0.09	0.001[a]
(95% CI)	(0.99 – 1.09)	(0.91 – 0.97)	
Change	-0.009 ± 0.076	-0.006 ± 0.090	-
p value	0.443[b]	0.691[b]	-
Uric acid (mg/dL)			
Baseline	3.81 ± 1.46	3.52 ± 1.34	0.382[a]
(95% CI)	(3.33 – 4.28)	(3.07 – 3.97)	
Week 12	3.75 ± 1.21	3.29 ± 0.91	0.065[a]
(95% CI)	(3.36 – 4.14)	(2.98 – 3.59)	
Change	0.05 ± 0.74	0.23 ± 0.91	-
p value	0.643[b]	0.125[b]	-

Variable	Control group	Experimental group	*p* value
AST (U/L)			
Baseline	20.59 ± 5.39	33.23 ± 58.14	0.196[a]
(95% CI)	(18.85 – 22.34)	(13.84 – 52.61)	
Week 12	23.50 ± 12.09	21.16 ± 4.71	0.276[a]
(95% CI)	(19.58 – 27.42)	(19.59 – 22.73)	
Change	-2.90 ± 12.16	12.06 ± 55.88	-
p value	0.145[b]	0.197[b]	-
ALT (U/L)			
Baseline	23.87 ± 10.02	28.28 ± 28.74	0.382[a]
(95% CI)	(20.62 – 27.13)	(18.69 – 37.86)	
Week 12	26.48 ± 12.20	21.00 ± 7.38	0.021[a]
(95% CI)	(22.53 – 30.44)	(18.54 – 23.46)	
Change	-2.61 ± 11.25	7.28 ± 25.39	-
p value	0.156[b]	0.090[b]	-
GGT (U/L)			
Baseline	27.02 ± 17.07	31.70 ± 27.35	0.371[a]
(95% CI)	(21.48 – 32.55)	(22.58 – 40.82)	
Week 12	30.92 ± 22.43	25.98 ± 12.66	0.245[a]
(95% CI)	(23.65 – 38.19)	(21.77 – 30.21)	
Change	-3.90 ± 16.95	5.71 ± 22.95	-
p value	0.159[b]	0.139[b]	-
ALP (U/L)			
Baseline	90.10 ± 22.16	88.04 ± 18.06	0.659[a]
(95% CI)	(83.92 – 97.28)	(82.02 – 94.06)	
Week 12	86.44 ± 20.84	89.97 ± 24.52	0.499[a]
(95% CI)	(79.68 – 93.19)	(81.79 – 98.14)	
Change	3.66 ± 12.89	-1.93 ± 18.60	-
p value	0.084[b]	0.531[b]	-

Values are expressed as mean ± standard deviation for normally distributed variables and median (25^{th} and 75^{th} percentiles) for non-normally distributed variables and 95% confidence interval (CI); TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; GL, glycemia; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; an Independent t test; b Paired t test.

DISCUSSION

According to the clinical variables (TJC28, TJC68, SJC28, SJC66, morning stiffness) and efficacy indicators (DAS28 (ESR)/(CRP), SDAI, CDAI, EULAR(ESR) / (CRP)response), supplementation with SBFO significantly alleviated joint pain, swelling, physical disability and stiffness in RA patients.

ESR and CRP are frequently used to detect and monitor systemic inflammation in routine clinical practice. CRP is a better marker of systemic inflammation compared to ESR. CRP is primarily produced in hepatocytes in response to proinflammatory cytokines (IL-6) whereas ESR is an indica-

tor of plasma level of acute phase proteins (16). At week 12, in SBFO group, CRP decreased while ESR increased compared to baseline values. This discrepancy might result from the fact that, in contrast to CRP, ESR level is also affected by factors that are not associated with chronic inflammation such as age, gender, body mass index, physical activity, common metabolic abnormalities, smoking and alcohol consumption (17).Our results are in agreement with previous clinical investigations reporting little impact of sea buckthorn fruit oil on CRP level. In a double-blinded, randomized, placebo-controlled crossover study (2 × 8 weeks, 4-week washout), sea buckthorn fruit oil supplementation (2000 mg/day) did not induce significant changes in plasma levels of CRP and other markers of inflammation (antitrypsin, orosomucoid, leukocytes). Sea buckthorn fruit oil was a commercially available product obtained from fruit flesh and seeds by supercritical carbon dioxide extraction (18). In obese children, sea buckthorn pulp oil (800 mg/day for 60 days) had weak effect on CRP (19).

In our study, SBFO supplementation (1800 mg daily, 12 weeks) did not significantly influence the lipid profile except HDL-C; the latter significantly decreased in SBFO group at week 12. The decrease in HDL-C in SBFO group was unexpected and might be attributed to the antihypertensive medications used by patients. Thus, 32.4% of patients in the experimental group were under treatment with nonselective β blockers for hypertension in comparison to only 5.1% of patients in the control group. The aforementioned medication has been reported to negatively impact the lipid profile by decreasing HDL-C and increasing TG (20-22). Previous studies investigating the impact of sea buckthorn oil on lipid profile reported inconsistent results. In mice fed a high-fat diet, sea buckthorn pulp oil lowered TG (serum, hepatic), TC (serum), LDL-C (serum, hepatic) but also HDL-C (serum, hepatic) (23). In healthy normolipidemic subjects, administration of sea buckthorn fruit oil obtained by supercritical carbon dioxide extraction (5 g/day, 4 weeks, crossover study) caused no change in plasma lipids (24). In obese children, sea buckthorn pulp oil (800 mg/day, 60 days) significantly improved plasma TC and TG but had weak effect on LDL-C and HDL-C levels (19). A significant increase in HDL-C was detected in atopic dermatitis patients supplemented with sea buckthorn pulp oil (5 g/day, 4 months) (25).

In the present study, at week 12, liver markers (AST, ALT, γ-GT) showed lower levels in SBFO group compared to control, with a significant reduction for ALT.Regarding other biochemical parameters, 12-week supplementation with SBFO (1800 mg daily) did not have a significant impact on ALP, GL, urea, creatinine and uric acid levels. Overall, SBFO was well-tolerated in patients with active RA.

CONCLUSIONS

The present study indicates beneficial effects of SBFO supplementation in active RA with significant improvements in composite disease activity indices and EULAR response. Taking into account boththe efficacy and safety of SBFO, the latter seems to be a promising option for the adjunctive treatment in RA. However, the impact of SBFO supplementation on

various inflammatory and oxidative stress markers needs to be further investigated. Different doses of SBFO should also be evaluated to determine the optimal dosage for the best therapeutic outcome.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest and this research received no external funding.

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