INFLUENCE OF BETALAIN-RICH EXTRACT ON REDUCTION OF DISCOMFORT ASSOCIATED WITH OSTEOARTHRITIS**

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Summary
Introduction. Osteoarthritis (OA) subjects typically experience progressive discomfort related to pain, joint stiffness, and general tiredness. The most common treatment of these conditions includes use of non-steroidal anti-inflammatory drugs (NSAIDS). However, efficacy of NSAID treatment is generally not completely satisfactory. Therefore, further improvements in management of OA-associated discomfort are needed.
Aim. The aim was to verify whether a betalain-rich red beet extract at dose range of 35-100 mg twice per day could reduce discomfort associated with osteoarthritis (OA) conditions.
Materials and methods. Study participants experiencing OA symptoms were treated with red beet extract (RBE) twice per day for exactly ten days. McGill and Energy Score data were evaluated at days 1, 5 and 10. The serum levels of advanced oxidation protein products (AOPP) were measured using a commercial kit (Cell Biolabs, Inc., #STA318).
Sera from volunteers treated with RBE were subjected to a cytokines and chemokines array as offered by Qynsys Inc.
Results. Collected data showed that ingestion of RBE for 10 days reduced McGill scores in a time- and dose-dependent manner with maximum 33% reduction as compared to the first day of the treatment. Interestingly, due to the treatment, serum levels of TNF-alpha were reduced in subjects whose serum TNF-alpha was greater than 1 pg/mL prior to initiation of the treatment. It was also found that serum levels of AOPP (proteins oxidized by hypochlorous acid/hypochlorites) were reduced by up to 48% after 10 days of the treatment.
Conclusions. This study showed that ingestion of RBE, at dosages greater than 35 mg, had a beneficial effect on pain associated with OA conditions. RBE may act by inhibiting protein oxidation typically induced by hypochlorous acid released from active neutrophils.

Key words: betacyanins; betalains; osteoarthritis; antioxidants; hypochlorous acid; chlorinated proteins

INTRODUCTION

Osteoarthritis (OA) subjects typically experience progressive discomfort related to pain, joint stiffness, and general tiredness (1, 2). The aetiology of this condition is complex and a number of factors are reported to contribute to development and progression of this condition (3). The most common treatments of these conditions include use of non-steroidal anti-inflammatory drugs (NSAIDS) (4). However, efficacy of NSAID treatment is generally not completely satisfactory. Therefore, further improvements in management of OA-associated discomfort are needed. Recently, it was reported by Steinbeck (5) that chlorinated peptides and elevated levels of myeloperoxidase (MPO) are associated with early OA conditions. Interestingly, Deberg published in 2008 a clinical observation showing that serum MPO was significantly reduced in OA patients after knee replacement (6). In 1991, it was shown by Katrantzis that the oxidant hypochlorite, a product of myeloperoxidase, degrades articular cartilage (7). In 2000, Bellometti followed changes in blood levels of nitric oxide, myeloperoxidase and glutathione peroxidase in OA patients subjected to mud baths. Interestingly, mud bath treatment was associated with reduced blood levels of MPO and nitric oxide (NO) (8). Altogether, these publications suggest a possible connection between OA conditions associated with damage of cartilage, and increased levels of chlorinated proteins and MPO.

Studies on OA treatment with natural formulations indicating a beneficial effect of ginger extract on the progress of osteoarthritis were recently discussed (9). Independently, red-violet betalains and yellow betaxanthins, the water-soluble nitrogenous pigments present in members of most families of the plant order Caryophyllales, were reported to reduce activity of

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neutrophil-derived myeloperoxidase. As described by Allegra *et al*. (10), betanin (one type of betalains) inhibits myeloperoxidase-mediated oxidation of low density lipoproteins and may also scavenge hypochlorous acid (11). Finally, in 2009, it was shown that beetroot juice may inhibit oxidative metabolism of neutrophils collected from obese subjects (12). Results presented recently (10-12) show that betalains may diminish activity of myeloperoxidase, resulting in reduced generation of hypochloric acid. Other independent research shows that betalains are well distributed in vivo after ingestion (13, 14). This characteristic favours the potential use of betalain-rich natural products for various health conditions associated with over-activation of neutrophils, and with involvement of myeloperoxidase and hypochlorous acid (as described above).

AIM

Following the above rationale, we hypothesized that ingestion of betalain-rich RBE containing 25% of total betalains may reduce general discomfort in subjects experiencing minor forms of OA. Consequently, this clinical exploratory study was designed to include measurements using the McGill scoring system and an Energy Score questionnaire in order to verify whether RBE may diminish OA discomfort. Study participants were divided into three experimental groups taking 35, 70 or 100 mg of RBE for 10 days only. Collected results are presented and discussed in this report.

MATERIALS AND METHODS

Betalain-rich red beet extract (RBE). A novel and proprietary food-based extract prepared from red beet roots was obtained from FutureCeuticals, Inc. USA, where it was produced using a patent-pending technology that does not require use of organic solvents and that significantly reduces amounts of sugar in the final material.

Betalain analysis. Quantification of betalains was performed by a spectrophotometric multiple-component method of Nilsson (15). Betalain profile analysis was performed according to Wybraniec (16).

Clinical study description and design. This study was designed to be an open type clinical discovery rather than a clinical efficacy study. The primary goal was to verify whether RBE may improve pain and fatigue associated with osteoarthritis conditions. The secondary goal of this study was to identify the minimum effective dose (MED). Therefore, we employed a multiple fixed-dose type study with three time points: day 1, 5, 10. There were 8 subjects per experimental group. Each group was treated with 100 mg (Group 1), 70 mg (Group 2) or 35 mg (Group 3) twice per day. All participants were asked to take one capsule of RBE 30 min prior to eating a meal.

Subjects for this study were selected randomly from a group of people who had been previously diagnosed with osteoarthritis and who had reported symptoms characteristic of OA such as joint pain, limited joint flexibility, and feeling energy-depleted due to chronic pain and joint problems. We used McGill pain score system and Energy Score system questionnaires at day 1, 5 and 10 as a means of quantifying symptoms.

Recruitment of subjects, treatments, blood sampling at day 1, 5 and 10, McGill and Energy Score tests and blood chemistry were performed by NutraClinical, Inc. (San Diego, California, USA). Measurements of human cytokines and chemokines in collected sera were provided by Quensys, Inc. on a research service basis. AOPP testing was performed on sera collected from subjects at day 1 and 10 days after the treatment in FutureCeuticals' lab using a commercially available kit (Cell Biolabs, Inc, USA).

RESULTS

Betalain composition. Red beet (*Beta vulgaris* L.) is commonly consumed as a food product and FutureCeuticals' RBE is a specially processed extract obtained from this material. Due to FutureCeuticals' RBE production process, this material is depleted of sugars and enriched in total betalains up to 24%. The relative betalain composition in RBE was approximately expressed by chromatographic signal values of main analysed pigments measured at their λ_{max}: betanin (12.6), isobetanin (15.6), 17-decarboxy-betanin (0.60), 17-decarboxy-isobetanin (0.44) and neobetanin (3.9).

Effect of RBE on pain feelings as measured using McGill test. This study was performed to verify our hypothesis that betalain-rich food-grade material RBE may reduce discomforts associated with painful and swollen joints in people suffering osteoarthritis.

Collected results show that all subjects reported reduced pain level in a dose-dependent manner as measured by using the McGill Questionnaire. Detailed data are provided in table 1. Following these data, it is clearly noticeable that treatment with RBE resulted in a significant improvement of the sensory part of the questionnaire.

Less improvement was observed for the affective aspects, and no effect was observed for the evaluative part of the questionnaire. Following this trend, treatment with 70 mg of RBE resulted in 41% reduction of pain as evaluated by the sensory part, but the total score for the McGill Questionnaire in this experimental group yielded a 33% reduction after day 10. It is also interesting to note that 5 days of treatment with 70 mg of RBE already resulted in 33% reduction of pain (total McGill score). This indicates that treatment with RBE may provide a moderately rapid effect (although not as acute as the effects of painkiller drugs such as NSAIDS). Exit interviews of study participants revealed that the first subjective improvements in pain were noticed after 3 days of the treatment. This observation strongly suggests that a 3-day period should be included in any future RBE clinical efficacy study protocol to follow rapid activity and effect on improvement of OA pain-related conditions.

Blood chemistry analysis. Standard serum biochemistry analysis was performed on each serum collected at day 1 and day 10. No unusual changes were noted in any parameters. All parameters were within the normal range (data not shown).

Table 1. Effect of RBE on McGill score after 5 and 10 days of treatment of OA subjects.

Group Dose	McGill Scores								
	Sensory Part			Affective Part			Evaluative Part		
	Day 1	Day5	Day 10	Day1	Day 5	Day 10	Day 1	Day 5	Day 10
Group 1-100 mg									
Average	58.87	41.00*	40.00*	4.12	3.62	2.25*	16.37	15.62	15.87
StD	3.31	4.40	7.21	1.80	2.19	1.48	3.42	4.83	4.45
Max	63	47	51	6	6	4	20.00	21.00	21.00
Min	53	35	30	1	1	1	10.00	8.00	8.00
StE	1.17	1.55	2.54	0.63	0.77	0.52	1.20	1.71	1.57
Suma	471	328	320	33	29	18	131	125	127
McGill Score	Day 1: 79.36; Day 5: 60.24; Day 10: 58.12								
Group 2-70 mg									
Average	59.62	44.37*	35.5*	2.25	2.00	1.75	15.50	13.37	14.75
StD	5.57	10.68	4.72	2.31	1.69	0.70	3.89	3.96	2.81
Max	68	63	46	6	6	3	23	19	18
Min	53	35	32	1	1	1	12	9	10
StE	1.97	3.77	1.66	0.81	0.59	0.25	1.37	1.40	0.99
Sum	477	355	284	18	16	14	124	107	118
McGill Score	Day 1: 77.37; Day 5: 59.74 Day 10: 52.00								
Group 3-35 mg									
Average	63.25	56.12	54.62	2.25	2.12	2.37	21.87	20.25	21.75
StD	7.02	9.86	9.59	0.70	0.83	0.51	2.85	3.28	3.80
Max	75	70	66	3	3	3	25	26	28
Min	55	37	35	1	1	2	16	16	17
StE	2.48	3.48	3.39	0.25	0.29	0.18	1.00	1.16	1.34
Sum	506	449	437	18	17	19	175	162	174
McGill Score	Day 1: 87.37; Day 5: 78.49; Day 10: 78.84								

[a]Sum of all values in the group

Subjective energy tests. In parallel to the McGill Questionnaire, all participants were required to answer 4 questions pertaining to their energy feeling rate (Q1), awareness rate (Q2), endurance rate (Q3) and mood rate (Q4). Rates for all these questions were scaled 1-4. This questionnaire (described in this article as Energy Score) was performed at day 1, 5 and 10. The highest number indicated a generally elevated level of the feeling rate.

All detailed data of this questionnaire are presented in Table 2. These data show that all participants reported feelings of increased awareness, energy, endurance and mood in a dose-dependent manner after treatment with RBE. Treatment with 70 mg resulted in 122% improvement over day 1 (tab. 2), whereas the treatment with RBE at a dose of 100 mg resulted in 81% improvement, indicating that treatment with the lower dose of 70 mg was optimal. Therefore, as was noted when analyzing the McGill data, the 70 mg dose seems to be the most potent for improvement of parameters listed in Table 2. Also, the lowest dose of 35 mg still provided significant Energy Score increases of up to 74% after 10 days of the treatment. In comparison, the McGill data for the same dose resulted in only 11% improvement. This may indicate that a primary effect of RBE is to modulate feelings of energy, mood, endurance and awareness, since a dose as low as 35 mg caused improvement of Energy Score up to 74%. These results were rather unexpected, since the energy score was

Table 2. Effect of treatment with RBE on Energy Score data.

	Day 1				Day 5				Day 10			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Group 1												
Average	1.14	1.28	1.28	1.42	1.42	2.14	1.71	2.48	2.14	2.28	2.57	2.57
StD	0.37	0.48	0.48	0.53	0.53	0.37	0.48	0.78	0.69	0.95	0.97	0.97
Max	2	2	2	2	2	3	2	4	3	4	4	4
Min	1	1	1	1	1	2	1	2	1	1	1	2
StE	0.14	0.45	0.45	0.20	0.20	0.14	0.18	0.29	0.26	0.35	0.36	0.36
Sum	9	9	9	10	10	15	12	17	15	16	18	18
Energy Score Average Sum +/- StD												
9.25+/-0.5					**13.5+/-3.1**				**16.75+/- 1.5 81% increase over day 1**			
Group 2												
Average	1.12	1.12	1.25	1.12	2.00	2.00	2.25	2.00	2.37	2.65	2.50	2.50
StD	0.35	0.35	0.46	0.35	1.30	1.06	0.88	0.00	1.40	1.18	0.92	0.92
Max	2	2	2	2	4	4	4	2	4	4	4	4
Min	1	1	1	1	1	1	1	2	1	1	2	2
StE	0.12	0.12	0.16	0.12	0.46	0.37	0.31	0.00	1.40	1.18	0.32	0.32
Sum	8	9	10	9	16	16	18	16	19	21	20	20
Energy Score Average Sum +/- StD												
9.0+/-0.81					**16.5+/- 1.0**				**20.0+/-0.8 1122% over Day 1**			
Group 3												
Average	1.14	1.28	1.28	1.57	1.57	1.42	1.71	2.14	2.00	2.14	2.42	2.14
StD	0.37	0.48	0.48	0.53	0.53	0.53	0.48	0.69	0.5	0.37	0.97	0.69
Max	2	2	2	2	2	2	2	3	3	3	4	3
Min	1	1	1	1	1	1	1	1	1	2	1	1
StE	0.14	0.18	0.18	0.18	0.20	0.20	0.18	0.26	0.21	0.14	0.36	0.26
Sum	8	9	9	11	11	10	12	15	14	15	17	15
Energy Score Average Sum +/- StD												
8.75+/- 1.5					**12.0+/-2.16**				**15.25+/-1.25 77% over Day 1**			

followed only as an additional subjective parameter to supplement the McGill pain score.

Analysis of advanced oxidation protein products. The serum levels of advanced oxidation protein products (AOPP) were measured using a commercial kit (Cell Biolabs, Inc., #STA318). This assay measures serum proteins modified by chloramine or hypochlorous acid. The detailed collected data are summarized in table 3. The data show a significant broad range (max and min) in baseline of AOPP at day 1 in each experimental group. Interestingly, this range was significantly reduced in each group after 10 days of the treatment. Resulting sum data show 36.3, 47.6 and 30.9% reduction in groups 1, 2 and 3, respectively. However, due to the broad range of AOPP values at day 1, StD is relatively high.

Cytokines and chemokines array. Sera from volunteers treated with RBE were subjected to a cytokines and chemokines array as offered by Qynsys Inc. Collected data showed that prior to treatment only 10 participants out of 24 were found to have TNF-alpha above the detection limit of 1 pg/mL per ELISA assay. However, treatment with RBE caused reduction of TNF-alpha in

Table 3. Effect of treatment with RBE on serum level of AOPP [ěM]

Result	Group 1		Group 2		Group 3	
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
Average	20.9	13.2	21.4	12.8	19.2	15.21
Std	14.9	7.2	12.4	7.3	8.0	4.9
Max	50.8	23.7	44.0	25.4	33.9	25.4
Min	7.8	6.8	8.7	5.0	8.5	10.2
Sum	144.6	92.2	171	89.7	154	106.5
% Reduction in average values						
	36.9		50.2		20.8	
% Reduction in sum values						
	36.3		47.6		30.9	

these 10 subjects after 10 days of the treatment (tab. 4). The same sera were additionally tested for changes in the levels of other cytokines and chemokines. This screening yielded data showing that treatment with RBE reduced serum level of IL6, GRO-alpha, and RANTES levels after 10 days of the treatment (tab. 4).

Table 4. Effect of RBE on blood level of selected cytokines and chemokines in subjects with TNF-alpha blood level >1 pg/mL. All other cytokines and chemokines are presented as concentration pg/mL. Upper number in row represents level of measured peptide at day 1, bottom number at day 10. DOD1 = change in peptide level at day 10 over day 1 and expressed as % of change.

1	2	3	4	5	6
Subject	**Group**	**TNF-alpha**	**IL-6**	**GRO-alpha**	**RANTES**
1	1	1.25 1.00	1.77 1.62	318.8 235.0	251442 218310
2		30.24 28.43	146.9 135.9	123.5 64.6	37542 27109
3		2.76 1.16	3.9 2.2	257.5 227.0	36127 33248
DOD1		**28.0%**	**23.7%**	**29.0%**	**16.6%**
N		3	3	3	3
4	2	3.05 1.42	ND	113.5 26.7	23800 10099
5		1.43 1.37	6.65 5.70	70.9 51.3	30582 23821
6		2.24 1.11	2.59 1.86	504.3 306.9	380997 211381
7		116.07 80.99	476.2 379.5	120.2 19.4	27932 25647
DOD1		**35.0%**	**22.0%**	**57.2%**	**33.7%**
N		4	3	4	4
8	3	1.341.12	4.73.3	73.3 63.9	15252 13115
9		1.701.61	2.21.2	48.5 32.4	36672 17600
10		172.35 171.88	293.2 268.5	135.2 116.5	15826 10265
DOD1		**8.3%**	**28.3%**	**21.0%**	**34.0%**
N		3	3	3	3

DISCUSSION

Data collected in the form of Energy and McGill scores show that RBE may indeed provide relief for conditions associated with OA. According to the working hypothesis mentioned at the beginning of this article, betalains may improve OA conditions due to their inhibitory effect on the chlorination of protein by hypochlorous acid released from activated neutrophils. This hypothesis was based on two rationales: 1) that betalains can reduce the amount of hypochlorous acid generated by activated neutrophils; and 2) that chlorinated proteins may contribute to onset of osteoarthritis and associated conditions.

In order to begin testing this hypothesis, the serum levels of AOPP were measured. The results presented herein are only indicative but justify further clinical investigation on OA subjects with increased serum AOPP levels.

AOPP is known as a pro-inflammatory factor and inducer of TNF-alpha release from monocytes. Therefore it was reasonable to verify whether treatment with RBE may result in reduction of blood TNF-alpha levels. In order to further investigate possible actions of betalains, sera from volunteers treated with RBE were subjected to a cytokines and chemokines array which indicated that RBE may have a favourable effect on blood levels of TNF-alpha. In addition, it was found that treatment with RBE also resulted in reduction of blood level of IL-6, RANTES, and GRO-alpha. Due to the rather limited number of participants per group showing serum level of TNF-alpha higher than 1 pg/mL, data in Table 4 are presented as indicative rather than definite. Further work on a higher number of OA subjects is required in order to confirm these observations and to understand any mechanisms of these effects.

It should be stressed that subjects with initial serum levels of TNF-alpha below 1 pg/mL also reported reduction of McGill score and improvements in Energy Score. This observation suggests that RBE may improve McGill and Energy scores in OA subjects in a TNF-alpha-independent manner. The investigators find this to be an intriguing observation that requires further research.

CONCLUSIONS

1. Betalains present in RBE may reduce the detrimental effect of hypochlorous acid released from active neutrophils in human subjects by inhibiting protein chlorination typically induced by hypochlorous acid.

2. Chlorinated proteins may contribute to onset of osteoarthritis and associated conditions.

3. These promising results are presented as new preliminary clinical observations that justify further clinical efficacy studies. □

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