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Silymarin (Livergol®) Decreases Disease Activity Score in Patients with Rheumatoid Arthritis: A Non-randomized Single-arm Clinical Trial

Mehrdad Shavandi[1], Ali Moini [2], Yadollah Shakiba[3], Ahmad Mashkorinia[2], Milad Dehghani[1], Shirin Asar[2], and Amir Kiani[4]

[1] Student Research Committee, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2] Department of Internal Medicine, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3] Regenerative Medicine Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4] Pharmaceutical Sciences Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease, which can lead to joint destruction and disability. Pannus formation due to chronic synovitis is the hallmark of RA. Oxidative stress as a consequence of immune cell activation and disease-modifying anti-rheumatic drugs can prevent inflammation and tissue destruction. Silymarin, an antioxidant extract from *Silybum marianum*, has been traditionally used for the treatment of liver diseases for decades.

In the present non-randomized single-arm clinical trial (NRSACT) study we evaluated the effects of silymarin tablet (Livergol®) on inflammatory markers in stable RA patients. Disease activity score (DAS-28) was measured before and after adding silymarin to standard drug regimen used for controlling inflammation in RA patients.

Silymarin significantly reduced the DAS28 related symptoms in 44 RA patients after 90 days (3.02±0.98 versus 2.3±0.74, $p<0.001$).

The exact mechanism of therapeutic effects of silymarin in RA patients is not clear but it could be as the results of its anti-inflammatory and anti-oxidative properties. Conducting the study on larger number of patients and also measuring cytokines levels including TNF-α and IL-1β may clarify the underlying mechanisms of the anti-inflammatory effects of silymarin in RA patients.

Keywords: Disease activity score; DAS 28; Rheumatoid arthritis; Silymarin

Corresponding Authors: Amir Kiani, PhD;
Department of Toxicology and Pharmacology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.Tel: (+98 918) 8316 929, E-mail: amir1kiani@yahoo.com, akiani@kums.ac.ir

Shirin Asar, MD;
Department of, Internal Medicine, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran. E-mail: sh758us@yahoo.com, shasar@kums.ac.ir

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INTRODUCTION

Rheumatoid arthritis (RA) is a complex, chronic, systemic autoimmune disease, which can lead to the destruction of the joints as well as disability. The survival rate in patients with RA is reduced by 3-10 years. The disease is characterized by swelling and stiffness of the joints that often occur in a symmetrical pattern on both sides of the body. The prevalence of RA is approximately one percent of the world's population (between 1.2 to 4.0%), and the incidence of it is mostly in the age of 60-40 years. RA is more common in females than males (3:1) and up to now there is no cure for it. Disease-modifying antirheumatic drugs (DMARDs) control the excessive inflammation in most patients but they also cause numerous side effect such as increasing free radical generation, and organs toxicity. In fact the life expectancy in patients with RA decreases by 3-10 years.[1,2]

In RA, there is a considerable immune cell infiltration in synovial cell membrane hyperplasia leading to production of large amounts of inflammatory cytokines and enzymes-including: TNF-α, IL-1β, IL-6, cyclo-oxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), matrix metalloproteinase-9 (MMP-9).[3-10]

An important clinical criterion in RA patients being widely used is disease activity score (DAS28), which determines the level of disease activity based on clinical as well as laboratory characteristics.[11] To determine this index, clinical variables like the number of tender and swollen joints and erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are measured qualitatively.[12] CRP is associated to ESR as an indicator of inflammation and its serum level decreases along with ESR after medical treatment, but it increases in the case of progression of symptoms.[13] DAS28 consisting of signs, symptoms, and paraclinacl tests(CRP , ESR) is generally accepted for evaluation of disease activity in RA patients.[12,14]

Targets for the treatment of RA patients include: controlling pain and swelling, procrastination of progress of the disease, reducing disability, and improving the quality of life.[15] To fulfill these purposes, medications such as acetaminophen, opioids, nonsteroidal anti-inflammatory drug (NSAIDs), glucocorticoid, DMARDs, and biologic drugs are used.[16,17] However, several studies have reported various side effects including; infections, gastrointestinal, and cardiovascular disorders for these medications.[18,19] Thus, finding efficient disease-modifying drugs with fewer side effects is critical for RA.[19]

Treatment with herbal medicines is one of the main components of complementary and alternative medicine, to which the public interest is growing.[20] Several studies have shown that, plant extracts that could modulate the expression of pro-inflammatory signals are particularly effective in controlling RA. These include flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins, and anthoxanthins, all of which are known to have anti-inflammatory effects.[21,22] Silymarin is extracted from *Silybum marianum* medicinal plant (milk thistle) and is traditionally used for the treatment of liver diseases.[23] Reports showed that silymarin promotes DNA polymerase, stabilizes cell membranes, inhibits free radicals, and increases glutathione levels, so that it can protect the liver against hepatotoxic agents.[24] Animal studies have indicated that silymarine inhibits lipo-oxygenase cycle, leukotrienes, and the formation of free radicals in Kupffer cells of mice, subsequently it may decrease the inflammation.[25] Another study on animal models found that silymarin has no side effects and its long-term use is safe.[26] Human studies have proven that in addition to the safety of using silymarin in adults, silymarin is safe to use even during pregnancy, breastfeeding and in children. The use of medicinal plants effective in the treatment of patients with RA in combination with synthetic drugs might reduce the necessary dose of synthetic drugs and in this way reducing the side effects of them. Moreover, previous studies revealed the anti-inflammatory properties of silymarin and considering no report on its side effects,[27,28] in the present study the effect of this plant extract on patients with RA was measured by assessing parameters like DAS28, ESR and CRP.

MATERIALS AND METHODS

This non-randomized single-arm clinical trial (NRSACT) was conducted by simple (convenient) non-random sampling method. This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences and registered in Iran registry of clinical trials (IRCT) with the number IRCT2013121915870N1.

In this study we selected 57 known stable RA patients under treatment with standard drug regimen of,

low dose prednisolone, hydroxy chloroquin or sulfasalazin, and methotrexate . Severe RA patients and new cases of disease were excluded, as it was possible that rheumatologists would change the drugs or doses during the time based on patient's condition. Our goal was to evaluate the efficacy of adding silymarin (Livergol, Goldaruo pharmaceutical, Iran) to the standard drug regimen in RA patients. The patients were selected from Rheumatology Clinic of Kermanshah University of Medical Sciences. All patients were diagnosed based on the 1987 American college of rheumatology (ACR) criteria and informed consent was obtained from all of them before the enrollment. The participants were known RA cases and were treated appropriately and followed for at least 2 year or longer. Patients were examined by a specialist rheumatologist and the number of tender, swelled joints was documented for disease activity score (DAS28) assay.

Baseline demographics and exact time of disease onset were asked from patients by face-to-face interviews. All patient were treated with total, 420 mg of silymarin daily, divided in, three doses for a 3-month.. The dose was determined according to similar studies that emphasized no risk for the patient.[29-31]

Among the volunteers, 13 patients left the study for different reason and ultimately 44 patients completed the treatment period of the study.

Laboratory Tests

10 mL venous blood sample was obtained from all fasting patients at both beginning as well as at the end of the study, and 5mL of sample was taken into test tubes and then centrifuged at 3000×g for 5 min. The collected serum was aliquoted and stored at −80°C until evaluation.

Westergren or manual method was used for ESR measurement and after one hour, the precipitated red blood cells were noted and the results was expressed as mm/h. CRP test was conducted with Bio kit (Werfen company , Barcelona, Spain).

The Severity of Disease Activity

To determine the level of disease activity, DAS was measured considering ESR, tenderness and swelling of 28 joints according to the following web site:
http://www.4s-dawn.com/products/rheumatology/das28-calculator/.

The patient's perception of pain was also included and the visual analog scale (VAS), a measure of converting qualitative variable of pain to a quantitative variable, was used. This criterion varies from zero to 100. In this scale, the patient gives 100 score to maximum amount of pain experienced by patient and zero score to the least pain (no pain).[32] Swelling and tenderness was also determined by one physician according to a Likert scale from zero to four scores, where the highest tenderness and swelling is considered as score 4.

Statistical Analysis

The collected information was analyzed using SPSS software version 16 (SPSS, Inc., Chicago, IL, USA). Kolmogorov-Smirnov test was used to test the normal distribution of data. Normal quantitative data was expressed as mean ± standard deviation and abnormal quantitative data as medium and interquartile range (IQR). To compare data for before and after the intervention, in case of normal distribution, paired t-test and in case of abnormal distribution, the Wilcoxon test was performed. The *p*-value of less than 0.05 was considered statistically significant.

RESULTS

In this study, of 44 patients who completed the course of treatment with the supplement drug (Livergol®), 13.6% were male and 86.4% were female. Their mean age was 47.59±12.64 with the range of 20 to 70 years old. According to Kolmogorov-Smirnov test, normality of parameter was examined and the only normal parameter was DAS28and other variables including swelling, tenderness, ESR, VAS, and CRP levels were non-normal. Then, the difference between before and after the intervention was measured in each variable. The results of swelling before and after the course of treatment, showed swelling reduction in 12 cases according to the specialist examinations (Table 1) and Wilcoxon test indicated this significant difference (p=0.001).

Also according to the specialist examinations, joints tenderness was reduced after treatment in 44 patients (Table 2) and statistical tests showed significant difference in the student T test results before and after drug administration (p=0.001). Pain conception was measured by VAS at the beginning and the end of treatment, as shown in Table 3. From patients'

perspective, in 41 cases the pain was reduced after the course of treatment. This difference also was statistically significant (p=0.001). The results of ESR laboratory tests showed that ESR was reduced in 26 cases and increased in 12 (Table 4).

Table 1. Changes in joint swelling before and after treatment with silymarin in patients with rheumatoid arthritis

Swelling	Before		After	
	Frequency	Percent	Frequency	Percent
0	29	65.9	34	77.3
1	6	13.6	5	11.4
2	4	9.1	5	11.4
3	3	6.8	0	0.0
4	2	4.5	0	0.0
Total	44	100.0	44	100.0

Table 2. Changes in joint tenderness before and after treatment with silymarin in patients with rheumatoid arthritis

Tenderness	Before		After	
	Frequency	Percent	Frequency	Percent
0	18	40.9	27	61.4
1	9	20.5	13	29.5
2	11	25.0	4	9.1
3	4	9.1	0	0.0
4	2	4.5	0	0.0
Total	44	100.0	44	100.0

Table 3. Changes in visual analog scale (VAS) before and after in patients with rheumatoid arthritis treatment with silymarin

VAS	Before		After	
	Frequency	Percent	Frequency	Percent
10	2	4.5	22	50.0
20	12	27.3	9	20.5
30	5	11.4	4	9.1
40	7	15.9	5	11.4
50	9	20.5	2	4.5
60	5	11.4	2	4.5
70	1	2.3	0	0.0
80	2	4.5	0	0.0
90	1	2.3	0	0.0
100	0	0	0	0.0
Total	44	100.0	44	100.0

Table 4. Changes in ESR in RA patient before and after treatment with silymarin in patients with rheumatoid arthritis

ESR	Before		After	
	Frequency	Percent	Frequency	Percent
15<	18	40.9	31	70.5
15-30	18	40.9	6	13.6
30-45	5	11.4	6	13.6
45=<	3	6.8	1	2.3
total	44	100.0	44	100.0

Statistical analysis showed significant difference in ESR before and after treatment (p=0.004). Results of CRP measurement for before and after supplement drug administration are also demonstrated in Table 5. As shown in this Table, 13 cases of reduction in CRP was observed, which was statistically significant (p=0.001). In this study, the severity of disease activity was measured with the DAS28 scale before and after the treatment. Before the start of treatment mean severity of disease activity was 3.0268±0.98806 and the difference was notably significant after the period of treatment, decreased as low as 2.3316±0.74472 (Tables 6 and 7). Statistical tests also confirmed significant difference in DAS28 variable before and after the treatment (p=0.001).

Table 5. Changes in CRP in in patients with rheumatoid arthritis before and after treatment with silymarin

CRP	Before		after	
	Frequency	Percent	Frequency	Percent
-	28	63.6	37	84.1
+	8	18.2	5	11.4
++	3	6.8	0	0.0
+++	4	9.2	0	0.0
missing	1	2.2	2	4.5
total	44	100	44	100.0

Table 6. Changes in disease severity score (DAS28) before and after treatment with silymarin in patients with rheumatoid arthritis

DAS	Before		after	
	Frequency	Percent	Frequency	Percent
2.6<	19	43.2	27	61.4
2.6-3.2	10	22.7	11	25.0
3.2-51	15	34.1	6	13.6
<5.1	0	0.0	0	0.0
total	44	100.0	44	100.0

Table7 . The level ofdisease severity score (DAS28) in patients with rheumatoid arthritis before and after treatment with silymarin

DAS 28	Before		After	
	Mean	Std. Deviation	Mean	Std. Deviation
Number of patient	3.0268	0.98806	2.3216	0.74472

DISCUSSION

RA is the most common systemic inflammatory joint disease with the prevalence of 1% worldwide. The disease can occur at any age, but the peak of incidence is between 40-60 years and disability is the common sign in this disease.[4,5] In this study, the case group had a mean age of 47.59±12.64 years (range between 10-70years) and most of them were female (86.4%), that is acceptable due to the higher incidence in females (3:1) than males.

Current RA medications including DMARDs as an important part of a common treatment plan cannot fully cure the disease. DMARDs just decrease the inflammatory symptoms and delay the progression of disease. They not only treat arthritis symptoms, but also

can postpone disease progressive joint destruction.

Studies have shown that, a combination therapy with two or more DMARDs is more effective than monotherapy; although it may have more side effects. MTX as the most commonly used DMARD has side effects as well; it can cause rash and stomach upset, toxicity for the liver and bone marrow, and birth defects.[33, 17-16] In addition to DMARDs, RA treatment medications may include NSAIDs and oral, intramuscular, or intravenous corticosteroids, but desirably these drugs should be used only for a short time because of the numerous side effects.[18,19]

Recent studies have found many natural compounds that have anti-arthritic potential.[34] Gamma linolenic acid (GLA), an unsaturated fatty acid in *Oenothera biennis* oils, seed of borage, and seed of cassis are known to be effective in reducing joint inflammation in patients suffering from RA.[35] Also, the final product of *Harpagophytum procumbens*, depending on the extraction process, has different fractions with agonistic, antagonistic, and synergistic anti-inflammatory properties.[36] Similar studies have been carried out on the *Ocimum*[37] and *Salix* species[38] indicating that they had some side effects beside the beneficial effects.[39,40]

Silymarin, the extract of milk thistle, is already widely used for certain liver diseases. Silymarin has properties such as strong antioxidant, decreasing liver lipids, effect on plasma lipids and lipoproteins, stimulating the formation of ribosomes, the cellular mRNA, and thereby tissue repair, anti-inflammatory, anti-cancer, anti-fibrotic, and anti-cirrhotic effects.[41] In various animal studies no toxicity or side effects was seen.[26,42] The main components of this drug are from the family of flavonoids, investigations on a variety of flavonoid compounds have observed only few side effects in animals and humans studies.[43] It also seems that there are no noticeable disturbing drug interactions for silymarin.[27,28] Considering these points,, anti-inflammatory effects of this drug on patients with RA were evaluated in the present study. Given that during the course of treatment, regimens for all patients was the same, and the effect of drugs of the patients' previous regimen had almost reached a steady state; therefore, all the changes in clinical symptoms were attributed to supplementary drugs. The results of the current study showed that administration of silymarin (Livergol®) as an adjuvant treatment in patients with RA, after a three-month period could significantly reduce disease symptoms such as swelling , tenderness, ESR, CRP, and pain from patients' PERSPECTIVE (i.e.VAS).

CRP and ESR can increase in RA disease.[12] As a result, the reduction of these two biomarkers confirms the effectiveness of this adjuvant drug. Silymarin anti-inflammatory mechanism is not well known, but it is thought to be related to inhibition of the transcription factor NF-κB. This factor plays an important role in regulating the expression of genes related to the inflammatory process.[44]

Silymarin also has considerable anti-inflammatory properties, Sodium salicylate and aspirin are known anti-inflammatory drugs that inhibit NF-κB. A previous study showed that silymarin is a more potent anti-inflammatory agent compared to NSIADS like aspirin, while having no side effects.[45] The results of this study indicated that after the course of treatment the severity of disease activity decreased significantly. Therefore, in general it can be mentioned that although treatment with pharmaciutical drugs is used commonly in RA, but these treatment methods have disadvantages including; high cost, long-term use, and side effects. There are also special considerations for women in fertility age, because many of these drugs have harmful effects in pregnancy. Consequently, physicians' and researchers' attention have recently been drawn to herbal medicines. These drugs have low or no side effect at usual doses along with good efficacy; also they have lower cost, compared to current medications. The present study showed that prescribing silymarin (Livergol®) can improve clinical and laboratory symptoms and have significant effects in reducing disease activity. Thus, further studies and trials regarding this herb.

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