Original Article

Iranian Journal of Immunology

https://iji.sums.ac.ir

The Effect of Oral Administration of Silymarin on Serum Levels of Tumor Necrosis Factor-α and Interleukin-1ß in Patients with Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid Arthritis (RA) is a systemic chronic autoimmune disease. Several inflammatory agents play key roles in RA pathogenesis, among which tumor necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β) are of great importance. Silymarin is a potent anti-oxidant extracted from Silybummarianum L. seeds.

Objective: To study the effect of silymarin on serum levels of TNF-α and IL-1β in patients with RA.

Methods: Patients with stable RA received 140 mg of silymarin, 3 times a day, for 3 months. Serum samples were collected before and after the treatment. Both TNF-α and IL-1β serum levels were measured by ELISA.

Results: 42 patients (14.3% male, and 85.7% female, with a mean age of 47.59±12.8 years old) completed the treatment course. There was no significant difference in the overall mean concentration of either TNF-α (P=0.14) or IL-1β (P=0.27) in all 42 patients after the treatment with Silymarin.

Conclusion: The addition of Silymarin to the treatment regimen of patients with stable RA has no significant effect on the serum levels of TNF-α and IL-1β, however, this study needs further evaluation with a larger sample size.

Keywords: IL-1β, Rheumatoid Arthritis, Silymarin, TNF-α

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Cite this article as:
Shavandi M, Yazdani Y, Asar S, Mohammadi A, Mohammadi-Noori E, Kiani A. The Effect of Oral Administration of Silymarin on Serum Levels of Tumor Necrosis Factor-α and Interleukin-1ß in Patients with Rheumatoid Arthritis. *Iran J Immunol.* 2022; 19(4):427-435, doi: 10.22034/iji.2022.90456.2007.

Received: 2021-03-11
Revised: 2022-05-10
Accepted: 2022-05-29

INTRODUCTION

Rheumatoid Arthritis (RA) is a systemic chronic inflammatory disease that inflicts damage upon multiple organs. However, it mainly targets peripheral joints and leads to destructive arthritis and non-suppurative, proliferative synovitis [1]. Although its etiology is still unclear, autoimmune mechanisms have a basic role in the disease pathogenesis and progress [2-4].

These mechanisms include inflammatory responses which result in the concentration of macrophages and other mononuclear cells (such as T cells) in the underlying layer of the synovium [5]. The expression of pro-inflammatory cytokines like Tumor Necrosis Factor-alpha (TNF-α) and Interleukin 1 beta (IL-1β) causes cascades that induce the activation of other immune cells [6]. Synovial fibroblasts produce various enzymes, such as collagenase and cathepsin, destroying joint matrix in response to IL-1β and TNF-α [5].

IL-1β and TNF-α also activate the nuclear factor-kappa B (NF-kB) pathway, which has an important role in RA pathogenesis and treatment [7]; this results in the expression of matrix metalloproteinase (MMPs) and irreversible destruction of joint, cartilage, and ligaments [8-11]. Several studies have shown that the blockage of the NF-kB pathway is a noteworthy therapeutic option [10, 12-14].

The desirable goals of RA treatment are mainly a reduction in pain intensity, decreasing joint destruction, improvement of physical function, and as a result, the prevention of disability [15, 16]. Four main categories of medication are implemented to accomplish these objectives: glucocorticoids, Disease-Modifying Anti-Rheumatic Drugs (DMARDs), Nonsteroidal anti-inflammatory drugs (NSAIDs), and analgesics [16]. Specifically, etanercept, infliximab, and adalimumab are administered to suppress TNF-α activity, and anakinra is employed to block the IL-1β pathway [17, 18].

Although these routine medications are potentially beneficial to improve RA signs and symptoms and to slow down the disease progression, they are accompanied by different adverse effects, such as serious infections, say, tuberculosis (TB), malignancies, adrenal insufficiency and osteoporosis [19-21]. These agents also have various considerable toxic effects on the liver, kidneys, hematopoiesis, and gastrointestinal system [16, 21, 22]. As a result, using alternative medicines with few side effects, such as herbal medications, has recently gained favor [23]. However, heterogeneity in response to the treatments for RA patients has been observed; it appears that while some patients benefit from a specific treatment regimen, others may not be as responsive to the same therapeutic strategy [24, 25].

Flavonoids, herbal complexes extracted from Silybum marianum L. seeds, have strong anti-inflammatory and antioxidant effects, especially in hepatobiliary diseases, as well as chemotherapy and radiotherapy damages [26, 27]. Asghar et al. suggested that the administration of silymarin as a dietary nutritional supplement may be useful in preventing free-radical-related diseases [28]. Furthermore, some studies suggest that silymarin could be effective as an anti-arthritic remedy in osteoarthritis [29, 30]. Apart from its anti-inflammatory properties, silymarin seems to have a broad spectrum of immunomodulatory functions which might be beneficial in protection against autoimmune diseases [31]. In an *in vitro* study conducted by Shariati et al. in 2019, silymarin was suggested to have immunoregulatory effects as it can promote regulatory T cell proliferation in multiple sclerosis (MS) patients [32]. Silibinin, a major active constituent of silymarin is assumed to display estrogen-like effects and bind estrogen receptors of immune cells. It is suggested that it can induce apoptosis, inhibit proliferation and reduce the expression of the pro-inflammatory cytokines, i.e. IL-17 and TNF-α, *in vitro*, via binding isolated T-cell receptors in RA patients [33]. In a human study, silymarin also could cause a significant decrease in disease activity scores in RA patients [34].

Although silymarin (contained in Livergol® tablets) is broadly used as a hepatoprotective agent with minimal adverse effects, mostly restricted to mild gastrointestinal upset [35-39], there are few studies investigating its effect on rheumatologic diseases. As silymarin is a natural antioxidant and its anti-inflammatory properties have been proven in various studies, it is expected to reduce the inflammatory mediators in immune-mediated diseases [40]. As a result, regarding the fact that TNF-α and IL-1β play important roles in RA pathogenesis [41], this study was conducted to investigate the effect of silymarin on serum levels of TNF-α and IL-1β in RA patients.

METHODS

Design

This before-and-after study was conducted at Kermanshah University of Medical Sciences, Kermanshah, Iran.

Setting

This study was conducted in the rheumatology clinic at Imam Reza hospital, Kermanshah, Iran. Patients' enrollment, intervention, and sample collection were performed from 07/09/2014 to 07/06/2015.

Enrollment of Subjects

After signing a written, informed voluntary consent, the subjects were enrolled according to the inclusion criteria as follows: Stable RA patients diagnosed by a rheumatologist, having 4 out of 7 items of ACR/EULAR (2010) Classification Criteria for RA [42, 43]. Exclusion criteria: Patients who had less than 2 years of RA history, any other background diseases, flare-up RA, and a history of previous consumption of silymarin, pregnant patients, as well as those who were putting biological medications to use.

Sampling

Subjects were selected using convenient sampling.

Intervention

After recording the demographic information and the exact time of the disease onset, all the patients received 140 mg of silymarin (Livergol® tablet, Goldaruo pharmaceutical, Iran) three times a day, for 3 months. The selected dosage was based on similar studies which guaranteed its safety [44].

Data Collection and Technique

The patients' standard treatment regimen included methotrexate, sulfasalazine, azathioprine, prednisolone, NSAIDs, alendronate, and calcium supplements. To measure the serum levels of TNF-α and IL-1β, a 5 mL venous blood sample was collected from all the volunteers before the intervention and after 3 months of Livergol use (baseline and endpoint of the study). All the patients fasted for 12 hrs. before each blood sample collection session. The samples were stored at -70°C before analysis. Both TNF-α and IL-1β titers were measured by Sandwich Enzyme-Linked Immuno-Sorbent Assay (ELISA) technique using the R&D Systems® ELISA Kit (USA), according to the manufacturer's instruction.

According to previous studies and considering the antioxidant and anti-inflammatory effects of silymarin, we expected that the serum levels of TNF-α and IL-1β would decrease in RA patients, who were included in the study under stable conditions. This strategy was used by us to lessen the impact of other interventional agents; however, the treatment did not meet our expectations, even though our previous study showed that patients were satisfied with the improvement in their life quality after taking silymarin. The current study also showed that although the health conditions were ameliorated post-treatment, it did not have a significant effect on the pattern of changes in TNF-α and IL-1β levels [24, 26, 27].

It should be mentioned that DAS-28, ESR, and CRP in these patients had already been

measured both before and after the treatment in another study conducted by Shavandi et al. [34]. We also collected the mentioned data and other clinical and biochemical parameters from the patients' files to evaluate probable associations between these factors. Measuring DAS-28 (disease activity score in 28 joints) is useful as it can indicate the response to a specific treatment since it is strongly associated with the levels of inflammation. It measures the overall disease activity on a scale of 0-10, based on the number of swollen and tender joints and acute-phase reactants like ESR and CRP.

Statistical Analysis

The collected data were analyzed using SPSS software Version 16.0 (Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was employed for the evaluation of the normal distribution of data and the Wilcoxon-signed rank test was performed to compare the data before and after the intervention. Pearson's correlation was employed to test the strength of relationships between the demographic information, the clinical and biochemical parameters before and after the administration of silymarin. The significance level was set at 0.05 in this study.

Ethical Issues

The design of the current study was approved by the Committee on Ethics in Research at Kermanshah University of Medical Science with the Ethics Committee Reference Number: 23322. This study was conducted according to the Declaration of Helsinki and was registered at www.irct.ir with the Registration Code: IRCT2013121915870N1.

RESULTS

The present investigation recruited 57 patients, 42 of whom (14.3 percent male and 85.7 percent female) finished the therapy course with a mean age of 47.60±12.8 years old and a range of 10 to 70 years old. The standard deviation was 12.80 (Table 1).

According to the Kolmogorov-Smirnov test, the distribution of data was abnormal; therefore, the Wilcoxon-signed rank test was carried out.

The results showed although the overall mean of both TNF-α and IL-1β concentrations in all the 42 patients had reduced, the difference was not statistically significant (P=0.14 for TNF-α; P=0.27 for IL-1β) (Figure 1).

It is noteworthy that Das-28, ESR, and CRP had shown a significant reduction in these patients after the treatment period in the previous study conducted by Shavandi et al. (P=0.001 for DAS-28; P=0.004 for ESR;

Table 1. Mean and Std. Deviation serum levels of TNFα, IL-1β, DAS-28, ESR, and CRP in patients before and after the treatment.

Parameter	Mean	Std. Deviation
Age	47.60	12.80
ESR before	20.88	18.03
ESR after	14.22	11.64
CRP before	0.52	0.93
CRP after	0.10	0.30
DAS-28 before	3.0354	1.02
DAS-28 after	2.3215	0.767
TNFα before	152.4	412.95
TNFα after	103.5	206.05
IL-1β before	40.73	122.27
IL-1β after	33.06	79.09

CRP, C-reactive protein; DAS-28, disease activity score 28; ESR, erythrocyte sedimentation rate; IL-1β, Interleukin 1 beta; TNF-α, tumor necrosis factor-alpha

P=0.001 for CRP). The association of serum levels of TNFα and IL-1β with these parameters in patients before and after treatment is shown in Table 2.

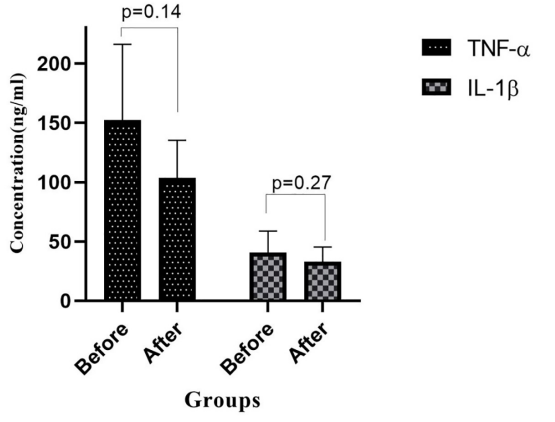

Figure 1. Comparison of the overall mean IL-1β and TNF-α levels before and after the treatment period in all patients.

No significant relation was observed between TNF-α and IL-1β with demographic and clinical data (Table 3).

DISCUSSION

This before-and-after study was conducted to investigate the effect of silymarin on serum levels of IL-1β and TNF-α in RA patients. The results showed heterogeneity in response to silymarin in the subjects. Our results demonstrated that the patterns of TNF- and IL-1 levels were not the same, with the majority of patients (64%) showing a decreased trend in their serum levels of TNF-α, while fewer

Table 2. Changes of IL-1β and TNF-α before and after the treatment period.

Rank		Count	Percentage (%)
TNF-α (After vs before)	Decrease	27a	64.2
	Increase	13b	31.0
	No change	2c	4.8
IL-1β (After vs before)	Decrease	14d	33.3
	Increase	25e	59.5
	No change	3f	7.2
a. TNF-α, after<TNF-α, before		b. TNF-α, after>TNF-α, before	
c. TNF-α, after=TNF-α, before		d. IL-1β, after<IL-1β, before	
e. IL-1β, after>IL-1β, before		f. IL-1β, after=IL-1β, before	

IL-1β, Interleukin 1 beta; TNF-α, tumor necrosis factor-alpha

Table 3. Association between serum levels of TNFα, IL1β, and DAS-28 with biochemical and clinical parameters in patients before and after the treatment

Parameters		TNFα		IL1β		DAS-28	
		Before	After	Before	After	Before	After
Gender	R[1]	0.181	0.192	0.119	0.113	-0.099	0.090
	P[2]	0.250	0.224	0.453	0.478	0.585	0.995
Age	R	0.101	0.115	0.081	0.071	0.291	0.181
	P	0.506	0.469	0.655	0.610	0.065	0.258
ESR before	R	-0.021	-0.028	0.380	0.023	0.634	0.590
	P	0.896	0.861	0.814	0.877	0.000	0.000
ESR after	R	-0.133	-0.142	-0.084	-0.089	0.357	0.627
	P	0.409	0.374	0.603	0.582	0.022*	0.000
CRP before	R	0.181	0.171	0.264	0.274	0.289	0.266
	P	0.269	0.290	0.094	0.099	0.087	0.097
CRP after	R	-0.083	-0.088	-0.068	-0.068	0.149	0.119
	P	0.613	0.594	0.679	0.683	0.366	0.471

CRP, C-reactive protein; DAS-28, disease activity score 28; ESR, erythrocyte sedimentation rate; ; IL-1β, Interleukin 1 beta ;TNF-α, tumor necrosis factor-alpha. [1]Correlation coefficient, [2]P-value

patients (33%) had a decreased trend in their serum levels of IL-1. The need for studies investigating immunological pathways and a broader population of patients may be advantageous in the treatment of RA because of the numerous intervening factors and silymarin's vast effects.

Silymarin is a potent antioxidant that has anti-carcinogenic activities, as well as hepatoprotective, and anti-fibrotic effects. Furthermore, it reduces plasma lipoprotein levels and can be beneficial in the treatment of chemotherapy-induced toxicity [27, 28, 45-47]. Wisher et al. suggested that 140mg of silymarin should be administered orally 2 or 3 times a day for hepatic diseases [48]. Silymarin increases the production of intracellular glutathione by controlling the activity of glutathione peroxidase; it also improves T cells' function by influencing reactive oxygen species [49-51]. In a study conducted by Ashkavand et al., combination therapy with silymarin and celecoxib in osteoarthritis patients decreased the required dosage of celecoxib and reduced the following complications [29]. On the other hand, previous studies have not shown any toxic effects in the therapeutic dosage of this agent, and no serious drug interactions have been mentioned [52, 53].

The molecular anti-inflammatory mechanisms of silymarin's impacts have not yet been fully fleshed out. However, according to some evidence, it is probable that the inhibition of NF-kB is responsible for a significant share of the anti-inflammatory effects of silymarin [54, 55]. NF-κB, a transcription factor, recognized as a major regulator for the expression of a wide variety of genes, is involved in inflammation, cytoprotection, and carcinogenesis. It specifically has an important role in the production of IL-1, IL-6, TNF-α, Interferon Gamma (IFN-γ), and Granulocyte macrophage-colony Stimulating Factor (GM-CSF) [54]. A study conducted in 2020, by Ziqiang Cui et al. showed that the administration of Retinoic Acid-Platinum complex in mice with RA can downregulate MEK/NF-κB pathway and TNF-alpha-induced inflammatory response [56]. In another study performed in 2019, the inflammatory processes in synovial tissue of mice with collagen-induced arthritis alleviated after using Taraxasterol (TAR); it was suggested that TAR blocks the activation of the NF-κB pathway, leading to the aforementioned results [57].

In a study conducted by Gharagozloo et al., TNF-α and neopterin significantly decreased in major β-thalassemia patients after 12 weeks of daily 420 mg silymarin application [58]. The anti-inflammatory activity of silymarin as well as its inhibiting effect on TNF-α production has been indicated *in vivo* and *in vitro* [59, 60]. Moreover, it has been suggested that silymarin inhibits both the cox-2 pathway and the production of interleukin-1 [61, 62]. Numan et al. indicated that using silymarin 300 mg daily alone or in combination with piroxicam or meloxicam can decrease inflammatory activity and pain intensity in osteoarthritis patients [63].

It is noteworthy that RA patients' response to different treatment strategies varies significantly. This means that while some patients greatly benefit from a given medication, others may have no response to it at all. As a result, it is recommended that personalized treatment regimens should be administered for each RA patient [24, 25].

Although a decrease in the concentration of IL-1β was seen in only 33.33% of patients in the current study, increasing the administered dosage of silymarin might increase the response rate in patients [52, 64]. To support this statement, the study conducted by Soon Kang suggested that the anti-inflammatory properties of silymarin and its inhibitory effects on the production of IL-1 and PGE_2 are dose-dependent [65].

In the current study, changes in two of the key mediators responsible for inflammatory responses in RA patients were evaluated. There are, however, other biochemical factors involved in the pathogenesis of RA; such as IL-4, IL-6, IL-10, and NFkB. Future

research should assess the likelihood of these parameters changing as a result of the silymarin administration.

CONCLUSION

The addition of silymarin to the treatment regimen of stable RA patients reduced TNF-α and IL-1β in 64.28 and 33.33% of the patients respectively. Additional research into this topic is recommended.

ACKNOWLEDGMENTS

This work was performed in partial fulfillment of the requirements for Pharm D. of Mehrdad Shavandi at the faculty of pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran. The design of this study was approved by the Committee of Ethics on Research at Kermanshah University of Medical Sciences (Ethics Committee reference number: 23322). The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 94147) for the financial support.

Conflict of Interest: None declared.

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