Anti-inflammatory activity of silymarin in patients with knee osteoarthritis

A comparative study with piroxicam and meloxicam

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ABSTRACT

الأهداف: لتقييم الفعل المضاد للالتهاب لمادة السليمارين لدى مرضى التهاب مفصل الركبة غير الرثوي OA مقارنة بالبيروكسيكام والميلوكسيكام.

الطريقة: أجريت دراسة سريرية عشوائية مزدوجة على 220 مريضاً (79 ذكر – 141 أنثى) بالتهاب مفصل الركبة غير الرثوي OA في شعبة أمراض المفاصل والروماتيزم – مستشفى بغداد التعليمي– بغداد – العراق، خلال الفترة مابين أكتوبر 2004م وحتى سبتمبر 2005م. تم تقسيم المرضى عشوائيا إلى خمسة مجاميع تم علاجها عن طريق الفم أما بمادة السليمارين (300mg) في اليوم، بيروكسيكام (20mg) في اليوم، ميلوكسيكام (15mg)، أو بإضافة السليمارين لكل من البيروكسيكام أو الميلوكسيكام ولمدة 8 أسابيع. تم قياس مستوى مصل الدم من المعايير البايوكيمياوية للالتهاب IL–1، IL–8، C3، C4 قبل لبدء بالعلاج وبعد ثمانية أسابيع.

النتائج: أدى العلاج بالسليمارين إلى خفض مستوى جميع المعايير التي تمت دراستها في مصل الدم مقارنتاً بفترة ما قبل العلاج، بينما خفض البيروكسيكام مستوى IL–8 فقط. أما بالنسبة للميلوكسيكام فقد أدى إلى زيادة في مستوى IL–1 بدون التأثير على المعايير الأخرى. وعند إضافة السليمارين مع البيروكسيكام انخفض مستوى جميع المعايير التي تمت دراستها، بينما لم تتأثر هذه المعايير عند استخدام السليمارين مع الميلوكسيكام.

خاتمة: أظهرت الدراسة تأثير السليمارين في خفض مستوى مصل الدم لبعض معايير الالتهاب مثل IL–1، IL–8، C3، C4 عند استخدامه منفرداً أو مضافاً إلى NSAIDs في علاج التهاب مفصل الركبة غير الرثوي OA.

Objectives: To evaluate the anti-inflammatory effect of Silymarin in patients with knee osteoarthritis (OA) in comparison with piroxicam and meloxicam.

Methods: A double-blind clinical trial was performed at the Department of Rheumatology, Baghdad Teaching Hospital, Baghdad, Iraq during the period from October 2004 to September 2005, in which 220 patients (79 males and 141 females) with painful knee osteoarthritis were randomized into 5 groups, treated with either silymarin (300mg/day), piroxicam (20mg/day), meloxicam (15mg), or a combination of silymarin with piroxicam or meloxicam. Serum levels of interleukin-1 alpha, interleukin-8, and the complement proteins C3 and C4 were assessed at zero time, and after 8 weeks.

Results: Silymarin reduces significantly serum levels of IL-1 alpha and IL-8, C3 and C4 after 8 weeks compared to the pre-treatment levels. Piroxicam showed no significant reduction in IL-1 alpha levels, while IL-8 decreased significantly, compared to pre-treatment value. Meloxicam elevates serum levels of IL-1 alpha significantly, while IL-8 did not significantly change compared to the pre-treatment value. Piroxicam or meloxicam produced slight, non-significant increase in serum levels of complement proteins after the 8-week treatment period. Adjunct use of silymarin with piroxicam results in significant reduction in both cytokines (IL-1 alpha and IL-8), and serum levels of C3 and C4. However, its adjunct use with, meloxicam did not reveal any significant changes in this respect.

Conclusion: Silymarin reduces the elevated levels of interleukins and complement proteins, when used alone, or in combination with NSAIDs for the treatment of knee OA.

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Inflammation is one of the most important processes involved in the defense of an organism, however, it often progresses to painful or chronically harmful diseases such as osteoarthritis (OA), and rheumatoid arthritis, needing pharmacological treatment. The inflammatory response involves many mechanisms that produce a multiplicity of vascular and cellular reactions, and many chemical mediators are involved in activating, and coordinating the various aspects of the inflammatory process.[1] Recent evidence indicates that, beside cyclooxygenase (COX)-activated products, a number of other mediators are involved in producing, and maintaining inflammation.[2] Leukotrienes contribute to the inflammatory response through a variety of effects, including an increase the migration of leukocytes, vascular permeability, and to produce diapedesis of the adherent leukocytes.[3] Many types of drugs exemplified by non-steroidal anti-inflammatory agents (NSAIDs) are currently being used to treat OA. However, NSAIDs elicit adverse effects particularly gastrointestinal ulceration.[4] Moreover, some of these agents have been reported to disrupt extracellular matrix metabolism, particularly proteoglycans synthesis.[5] The need for more effective treatment of arthritis with fewer side effects has encouraged the search for complementary or alternative approaches, and has attracted the interest of clinicians, as well as patients.[6,7] Several complementary alternative medicine products derived from herbs have been reported to act as anti-inflammatory agents. Curcumin, extracted from the rhizomes of *Curcuma longa L.* (Zingiberaceae) has been used in Asia for the treatment of inflammatory disorders.[8,9] Many investigators have proven that a variety of flavonoid molecules including silymarin, the extract from the ripe seeds of Silybum marianum, possess anti-inflammatory activity on various animal models of acute, and chronic inflammation.[10-12] In addition to so many diverse pharmacological activities and antioxidant properties demonstrated by silymarin, several studies have demonstrated a variety of other anti-inflammatory effects, including mast cell stabilization,[13] inhibition of neutrophils migration,[14] inhibition of leukotriene synthesis, and prostaglandin formation.[15,16] Thus, it may be valuable to continuously evaluate the anti-inflammatory activity of flavonoids, not only for establishing anti-inflammatory mechanism, but also for developing a new class of anti-inflammatory agents.[11] The main objective of this study was to assess the anti-inflammatory effect of silymarin in patients with knee OA, when used alone, or in combination with NSAIDs.

Methods. This double-blind clinical study was performed on 220 patients (79 males and 141 females), with an age range of 38-75 years (53.07± 8.18) with painful knee OA, at the outpatient clinic in the Department of Rheumatology, Baghdad Teaching Hospital, Medical City, Baghdad, Iraq during the period from October 2004 to September 2005. The protocol was approved by the ethical committee of the College of Pharmacy, University of Baghdad, Baghdad, Iraq. All patients have symptomatic and radiologic evidence of OA in one or both knee joints; their clinical features were in accordance with the description of OA in the United Kingdom and North American Clinical Guidelines. Patients were included in the study if they met the following criteria: agreed to participate in the study and signed the informed consent form, diagnosed with OA in one or both knee joints, not taking any kind of antioxidant vitamins or anti-inflammatory agents for at least one month, and have no other evident medical problem. Subjects were excluded from the study if they were unable to provide informed consent, had current significant illness, or use drugs for treatment for inflammation, and those who miss taking medication for more than 2 days. After obtaining their signed consent, patients were allocated into 5 groups: group A include 20 patients treated with silymarin capsule (300 mg/day), specially prepared from a standard crude extract received as a gift from Luna Company, Egypt, given in 2 divided doses for 8 weeks, group B include 50 patients treated with 20 mg/day piroxicam capsule (Medico, India) given at night for 8 weeks, group C include 50 patients treated with a combination of piroxicam and silymarin in doses and duration as indicated above, group D included 50 patients treated with single daily doses of 15 mg meloxicam tablets (Ajanta Pharma Limited, India) given at night for 2 weeks, and group E include 50 patients treated with a combination of silymarin and meloxicam as indicated previously. Out of these 220 patients, only 167 patients completed the study.

Blood samples (10 ml) were collected from the veins of each patient, before starting the treatment, and after 8 weeks in a plain tube, and left for clot formation. Serum was separated by centrifugation at 5000 rpm for 10 minutes and stored frozen at -18°C until analyzed. The serum was analyzed for interleukin (IL)-1α, and IL-8, using ready made enzyme-linked immunosorbent assay (ELISA) kits (Immunotech, France) specific for human interleukins,[17,18] and the complement proteins, C3 and C4, using radial immunodiffusion plates obtained ready made for this purpose (Biomaghreb, Tunisia).[19] The mean values of all parameters were expressed with SEM, student's t-test, and analysis of variance were used to check their significance, and considered significant at $p<0.05$.

Results. Table 1 clearly indicated that treatment of OA patients with silymarin (300 mg/day), orally reduces significant serum levels of the cytokines IL-1α (56%), and IL-8 after 8 weeks (58%), p=0.02, compared to pre-treatment levels. Meanwhile, piroxicam (20 mg/day) did not show significant reduction in IL-1α levels, while IL-8 was reduced by 15%, and significantly different compared to the pre-treatment value. In case of treatment with meloxicam (15 mg/day), serum level of IL-1α was significantly elevated (47%, p=0.01) compared to the pre-treatment value, while IL-8 did not significantly change (**Table 1**). The adjunct use of silymarin with piroxicam results in a significant reduction in both cytokines (IL-1α [42%], and IL-8 [56%], p=0.01) compared with the pre-treatment values after 8 weeks. However, its adjunct use with meloxicam did not reveal any significant changes in the serum levels of the measured cytokines. When the results of all groups statistically compared using analysis of variance, significant differences were found in the effect of different treatment modalities on IL-1α levels only (**Table 1**). **Table 2** shows that treatment of OA patients for 8 weeks with silymarin significantly reduced the serum levels of the complement proteins, C3 (81%) and C4 (45%), compared to the pre-treatment levels. However, piroxicam or meloxicam produced slight, non-significant increase in the serum levels of both complement proteins after 8 weeks treatment period. The adjunct use of silymarin with piroxicam improves the effect of the later, significantly reducing serum levels of C3 (18%) and C4 (47%), p=0.02, compared to the pre-treatment values, while adjunct use of silymarin with meloxicam did not significantly change the serum levels of C3 and C4 during the same period of treatment. The analysis of variations with analysis of variance indicates that significant differences were observed within the C3 values in this respect (**Table 2**).

Table 1 - Effect of treatment wit Silymarin alone or in combination with Piroxicam or Meloxicam on serum levels of IL-1α and IL-8 in patients with knee osteoarthritis (OA).

Treatment group	Serum IL-1α (pg/ml)		*P* value	Serum IL-8 (pg/ml)		*P* value
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
Silymarin 300mg/day (n=20)	358.4 ± 45.9	156.8 ± 19.6^{*a}	0.02	386.9 ± 52.4	162.3 ± 48.3^{*a}	0.02
Piroxicam 20mg/day (n=35)	314.7 ± 70.7	304.9 ± 67.8^{b}	0.07	264.8 ± 63.4	208.3 ± 45.4^{*b}	0.03
Piroxicam 20mg/day + Silymarin 300mg/day (n=40)	296.8 ± 53.1	170.8 ± 18.2^{*a}	0.02	488.9 ± 7.9	213.3 ± 39.1^{*b}	0.02
Meloxicam 15mg/day (n=32)	242.3 ± 47.6	356.9 ± 45.1^{*b}	0.01	301.3 ± 87.0	370.9 ± 87.7^{c}	0.06
Meloxicam 15mg/day + Silymarin 300mg/day (n=40)	248.3 ± 39.7	205.4 ± 28.5^{a}	0.07	246.5 ± 79.5	312.3 ± 87.0^{c}	0.05

Data are expressed as mean ± SE. n= number of patients, * significantly different compared to pre-treatment values (p<0.05), values with non-identical superscripts (a, b, c) within the same parameter are considered significantly different (p<0.05).

Table 2 - Effect of treatment wit Silymarin alone or in combination with Piroxicam or Meloxicam on serum complement proteins C3 and C4 in patients with knee osteoarthritis (OA).

Treatment group	Serum IL-1α (pg/ml)		*P* value	Serum IL-8 (pg/ml)		*P* value
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
Silymarin 300mg/day (n=20)	207.2 ± 9.8	39.34 ± 12.2^{*a}	0.001	78.8 ± 7.4	43.5 ± 6.7^{*a}	0.01
Piroxicam 20mg/day (n=35)	163.5 ± 7.6	166.1 ± 16.1^{b}	0.07	35.8 ± 2.0	44.8 ± 11.7^{a}	0.06
Piroxicam 20mg/day + Silymarin 300mg/day (n=40)	189.4 ± 27.6	155.1 ± 14.1*b	0.02	59.1 ± 8.5	31.1 ± 5.1^{*b}	0.01
Meloxicam 15mg/day (n=32)	131.8 ± 10.6	186.2 ± 28.2^{c}	0.05	44.0 ± 6.3	49.0 ± 9.1^{a}	0.07
Meloxicam 15mg/day + Silymarin 300mg/day (n=40)	115.2 ± 10.3	121.9 ± 17.6^{b}	0.06	50.7 ± 9.1	41.9 ± 6.0^{a}	0.05

Data are expressed as mean ± SE. *significantly different compared to pre-treatment values (p<0.05), values with non-identical superscripts (a, b, c) within the same parameter are considered significantly different (p<0.05).

Discussion. Synovial tissue synthesizes mediators of inflammatory cascade in the joint, which plays an important role in the pathophysiology of OA. These include tissue pro-inflammatory mediators such as IL-1α, IL-1α, IL-4, IL-6, and TNF-α, in addition to prostaglandin, chemokines, tissue inhibitors of metalloproteinase, and COX.[20] Once liberated, IL-1α stimulates the release of a cascade of cytokines including TNF-α and TGF-β. These cytokines induce chondrocytes to release lytic enzymes, including metalloproteinases, which degrade collagen II and proteoglycans, and inhibit normal matrix synthesis by chondrocytes.[21] These biochemical changes in the cartilage matrix decrease its tensile strength and resiliency, preventing it from functioning normally in transmitting forces, supporting chondrocytes, and protecting sub-chondral bone. Interleukin-1 also increased the expression of phospholipase A2 in rabbit chondrocytes, so that substrate availability for prostaglandin synthesis was increased in the joint.[22] It induces joint articular cells, such as chondrocytes and synovial cells, to produce other cytokines such as IL-8, IL-6, as well as stimulate proteases, and PGE2 production. Interleukin-8 is a potent chemotactic cytokine for polymorphonuclear neutrophils (PMNs), stimulating their chemotaxis, and generating reactive oxygen metabolites.[23] Deleuran et al[24] reported strong expression of IL-8, in both OA, and RA patients detected in the blood vessels, and lining the cell layers of the selected synovial membrane. Interleukin-8 was also present in the chondrocytes, and has been shown to enhance the production of oxidative and 5-lipoxygenase (5-LO) Products.[25] The data presented in this study clearly demonstrated that treatment with silymarin significantly reduces the serum levels of IL-1α and IL-8 after 8 weeks compared to pre-treatment levels. The anti-inflammatory action of silymarin, through the inhibition of IL-1α and prostaglandin E2 (PGE2), was reported in a dose-dependent manner in isolated mouse peritoneal macrophages, and RAW264-7 cells,[26] and may explain the finding reported in this study. Meanwhile, treatment of OA patients with piroxicam for 8 weeks did not show significant reduction in the IL-1α levels. However, IL-8 was reduced by 15%, a value that was significantly different compared to the pre-treatment values. The treatment with meloxicam for the same period resulted in significant elevation in serum levels of IL-1α, compared to the pre-treatment values, but IL-8 level was not significantly changed (**Table 1**). Inhibition of COX-enzymes by NSAIDs leads to shunt the arachidonic acid metabolism towards the 5-LO pathway, which may increase formation of Lt's,[27] and the enhancement of leukotrien B4 (LTB4) synthesis was documented in OA and RA patients receiving NSAIDs for more than 3 months. Moreover, a shunt effect has also been demonstrated in vitro on human osteoblasts from sub-chondral bone, and it has been demonstrated that long-term treatment of cells with a COX-2 specific inhibitor (NS-398, 10 μM) increased the level of LTB4 4-fold, and interestingly, the PGE2 levels were no more reduced, than after a short-term treatment.[28] Concerning the influence of the immune system in this respect, excessive release of LTB4 stimulates the production and release of pro-inflammatory cytokines from macrophages, lymphocytes, and synovial membrane. Neither selective, nor non-selective NSAIDs are found effective against LT's,[29,30] a situation that makes interference with the multiple inflammatory pathways impossible, and mark such therapeutic approach no more than simple analgesia, without modification in the pathophysiology of the disease. The dual inhibitory effects of silymarin on COX's and 5-LO pathways will produce a wider spectrum of anti-inflammatory effects, and reduce the production of different pro-inflammatory mediators.[31] Similarly, dual inhibition of COX's and 5-LO may limit the vascular changes seen during inflammation, and leukocyte-induced gastrointestinal damage. In this study, meloxicam, which is to some extent considered more selective for COX-2,[32] significantly elevated serum IL-1α after 8 weeks of treatment at a dose level of 15 mg/day, and this result seems consistent with that observed in the previously mentioned report. Other studies concerning the effects of many flavonoids belonged to the same chemical class of silymarin, such as quercetin, and rutin, when administered intraperitoneally suppress lethal endotoxic shock induced by lipopolysaccharide, or lipopolysaccharide plus D-galactosamine in mice, in addition to that, rutin effectively reduces TNF-α production.[33,34] Whether flavonoids from daily food intake really affect an inflammatory response is not clearly established.[9,11] No clinical data showing the relation between flavonoid intake and incidence or severity of inflammatory disorders, such as RA and OA was available. Accordingly, the exact therapeutic dose of silymarin cannot be exactly characterized, and this is considered an important limitation of the study. On the other hand, the pharmacological effect produced by the flavonoid is quite different, and treatment with a certain flavonoid may affect, at least in part, some inflammatory responses in many clinical situations.[35,36]

In this study, **Table 2** showed that treatment of OA patients with silymarin for 8 weeks results in significant reduction in serum levels of C3 and C4, compared to the pre-treatment levels, which meant that interference with the amplification cascade of inflammation is one of the mechanisms through which silymarin may produce its anti-inflammatory activity. However, treatment of OA patients with piroxicam or meloxicam alone did

not show significant changes in serum levels of both complement proteins after 8 weeks, while adjunct use of silymarin with piroxicam succeeded in reducing C3 and C4 serum levels significantly. These results are comparable with those observed with quercetin, another potent flavonoid that prevents further recruitment of inflammation cells to the site of inflammatory response.[37] All these including the present study, proved that several flavonoids including silymarin really inhibit the expression of pro-inflammatory molecules in experimental animal models and human studies. These findings suggested that modulation of pro-inflammatory mechanisms is certainly one of the major actions, or mechanisms of flavonoids that may explain their anti-inflammatory activity. Unlike NSAIDs, these modulating activities are unique, and newly observed for the anti-inflammatory flavonoids. However, to establish clearly the in vivo effect in this respect, various flavonoids should be examined in clinical trials in many inflammatory disorders. In conclusion, silymarin effectively reduces the elevated serum levels of IL's, and C3 and C4, when used alone, or in combination with NSAIDs for the treatment of knee OA.

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