

## Therapeutic Use of Silymarin in the Management of Suspected Renal and Hepatic Injury Produced by NSAIDs in Osteoarthritis Patients

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### Abstract :

Long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) mostly associated with renal and hepatic adverse effects, and the adjunct use of compounds with potent protective effects, like silymarin, may be one of the choices to avoid these effects. This project was designed to evaluate the protective effect of silymarin against the suspected renal and hepatic injury induced with long term use of NSAIDs; 220 patients with osteoarthritis were randomized into 5 groups and treated with either silymarin 300mg/day alone, piroxicam 20mg/day alone, meloxicam 15mg/day alone or the combination of each of them with silymarin for 8 weeks. The renal and hepatic functions were evaluated before starting treatment and after 8 weeks including assessment of serum levels of urea, creatinine and the activities of the hepatic enzymes alkaline phosphatase (ALP), glutamic-oxalic acid transaminase (GOT) and glutamic-pyruvic acid transaminase (GPT). The results indicated that using NSAIDs alone produced elevation in the markers of renal and hepatic damage that can be successfully prevented or reversed when silymarin adjunctly used with them. In conclusion, silymarin when co-administered with the NSAIDs (piroxicam or meloxicam) decreases their renal and hepatic toxicities in OA patients.

**Key words:** Silymarin; Piroxicam, Meloxicam; Nephroprotection; Hepatoprotection

### الخلاصة

دائماً ما يتسبب الاستخدام المزمن لمضادات الالتهاب غير الستيرويدية في حدوث أضرار جانبية في الكبد والكليتين، وإن استخدام مركبات لها القدرة على حماية الجسم ضد هذه التأثيرات، مثل السليمارين، قد يكون من ضمن الخيارات المتاحة والتي يمكن اعتمادها لمثل هذا الغرض. تم تصميم هذه الدراسة لمعرفة تأثير السليمارين في الوقاية من التأثيرات الجانبية المتوقعة لمضادات الالتهاب غير الستيرويدية على الكبد والكليتين. أجريت الدراسة على 220 مريضاً مصاباً بالتهاب المفاصل غير الرثوي وتم توزيعهم عشوائياً على خمسة مجاميع وعلاجهم إما بمادة السليمارين فقط (300 ملغم/يوم) أو بيروكسيكام فقط (20 ملغم/يوم) أو ميلوكسيكام فقط (15 ملغم/يوم) أو بإضافة السليمارين إلى كل منهما ولمدة 8 أسابيع. تم فحص وظائف الكليتين والكبد قبل البدء بالعلاج وبعد 8 أسابيع، وتضمنت في GOT, GPT, ALP هذه الفحوصات قياس مستوى مانتي اليوريا والكرياتينين في مصل الدم وكذلك فعالية الأنزيمات الكبدية في مصل الدم. أظهرت نتائج الدراسة إن استخدام البيروكسيكام والميلوكسيكام لوحدهما يمكن أن يؤدي إلى رفع مستوى المعايير البيولوجية لوظائف الكلية والكبد والتي يمكن الحد منها بإضافة السليمارين إلى النظام العلاجي. يمكن الاستنتاج بأن السليمارين عندما يستخدم مع مضادات الالتهاب غير الستيرويدية يمكن أن يحد من أضرارها الجانبية على الكليتين والكبد لدى مرضى التهاب المفاصل غير الرثوي.

### Introduction :

Many physiological functions other than inflammation are reported for prostaglandins (PGs) including maintenance of gastromucosal integrity,<sup>(1)</sup> modulation of renal microvascular hemodynamics and tubular salts and water reabsorption.<sup>(2)</sup> So, nephrotoxicity, gastropathy and other forms of tissue injury are reported as a result of long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) ( both selective and non-selective cyclooxygenase inhibitors),<sup>(3)</sup> and considered as an important parameter during long-term therapy.<sup>(4)</sup> According to the available reports, all NSAIDs, selective and non-selective COX inhibitors, share relatively the same therapeutic and adverse effects profile, and caution should be exercised with

their use; meanwhile, adjuvant cytoprotection should be considered.<sup>(5,6)</sup> Silymarin is a mixture of flavolignans isolated from the ripe seeds of the medicinal plant *Silybum marianum* (Milk thistle).<sup>(7)</sup> It has many pharmacological activities including anticancer,<sup>(8)</sup> hepatoprotective,<sup>(9)</sup> cardioprotective,<sup>(10)</sup> nephroprotective,<sup>(11)</sup> and neuroprotective<sup>(12)</sup> activities that mostly attributed to its powerful antioxidant properties and the ability to modulate responses of different cellular receptor systems.<sup>(13, 14)</sup> The present study was designed to evaluate the protective effects of silymarin against the suspected renal and hepatic injury that might induce by long-term use of NSAIDs in patients with osteoarthritis (OA).

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## Patients and Methods :

This study was performed on 220 patients (79 males and 141 females) with painful knee osteoarthritis at the outpatient clinic in Baghdad Teaching Hospital, with an age range of 38-75 years ( $53.07 \pm 8.18$ ). 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 United Kingdom and North American Clinical Guidelines. After obtaining their signed consent, patients were allocated into five groups: group A included 20 patients treated with Silymarin capsule (300mg/day), specially prepared from a standard crude extract (received as a gift from Luna Company, Egypt) given in two divided doses for eight weeks; group B included 50 patients treated with 20mg/day Piroxicam capsule (Medico, India) given at night for eight weeks; group C included 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 15mg Meloxicam tablets (Ajanta Pharma Limited, India) given at night for two weeks and group E included 50 patients treated with a combination of Silymarin and Meloxicam as indicated previously. Only 167 patients completed the study. Blood samples (10ml) were collected from the veins of each patient, before starting treatment and after 8 weeks, in a plain tube and left for clot formation. Serum was separated by centrifugation at 5000 rpm for 10 min and stored frozen at  $-18^{\circ}\text{C}$  until analysed. The serum was analysed for urea and creatinine levels as markers of renal function, and the activities of the aminotransferases (ALT, AST) and alkaline phosphatase (ALP) according to standard methods.<sup>(15, 16, 17, 18)</sup> The mean values of all parameters were expressed with SEM; Student's *t*-test and ANOVA were used to check their significance, and considered significantly different at  $P < 0.05$ .

## Results :

The results presented in table 1 indicated that although serum levels of urea and creatinine are within normal values, treatment with (300mg/day) silymarin for 8 weeks resulted in significant reduction in the serum levels of both parameters (22% and 26%

respectively) compared to pre-treatment value. Meanwhile, treatment of other group of OA patients with piroxicam for 8 weeks significantly elevated serum urea level (14%), while serum creatinine seems to be not significantly affected. However, treatment with meloxicam for the same period causes significant elevation in both, serum urea and creatinine levels (8% and 10% respectively) compared to pre-treatment values. Combination of the NSAIDs with (300mg/day) silymarin results in significant reduction in the serum urea (16 and 19% respectively), and serum creatinine (29% and 39% respectively) compared to pre-treatment values (Table 1). Analysis of inter group variations using ANOVA showed that significant differences among groups were detected in this respect ( $P < 0.001$ ) for both parameters. The data presented in (table 2) clearly showed that although serum activities of the hepatic enzymes, alkaline phosphatase (ALP), glutamic acid oxaloacetate transaminase (GOT) and glutamic pyruvic acid transaminase (GPT) are within the normal values in all groups. Treatment of OA patients with silymarin significantly reduces serum enzymes activity (25%, 15% and 20% respectively) compared to pre-treatment values. Table 2 also showed that treatment with piroxicam for 8 weeks resulted in significant elevation in the serum activities of ALP and GPT (13% and 57% respectively), while GOT activity did not significantly affected. Meanwhile, treatment with meloxicam for the same period in other group of OA patients resulted in significant increase in ALP activities in serum (17%) while the activities of the other two enzymes showed non-significant increase only within the same period of treatment. Combination of both NSAIDs used with 300mg/day of silymarin resulted in significant reduction in the activities of liver enzymes in the serum after 8 weeks, where ALP activity significantly reduced by 20% and 24% respectively, GOT activity was reduced by 28.5% and 50% respectively, and GPT activity was reduced by 33% and 35% respectively compared to the pre-treatment values. Analysis of inter group variations using ANOVA revealed significant difference among groups in this respect ( $P < 0.001$ ) for all parameters.

Table 1. Effects of silymarin on the serum levels of urea and creatinine in OA patients treated with piroxicam or meloxicam.

Treatment group	Serum urea (mmol/l)		Serum creatinine (mmol/l)	
	Pre-treatment	post-treatment	pre-treatment	post-treatment
Silymarin (n =20)	4.81 ± 0.21	3.75 ± 0.08* <sup>a</sup>	61.00 ± 5.30	45.1 ± 1.77* <sup>a</sup>
Piroxicam (n =35)	4.43 ± 0.16	5.06 ± 0.22* <sup>b</sup>	65.42 ± 3.54	61.9 ± 2.65 <sup>b</sup>
Piroxicam + Silymarin (n =40)	4.92 ± 0.28	4.14 ± 0.16* <sup>c</sup>	69.84 ± 4.42	49.5 ± 2.65* <sup>c</sup>
Meloxicam (n =32)	4.74 ± 0.22	5.12 ± 0.23* <sup>b</sup>	54.81 ± 2.65	60.1 ± 2.65* <sup>b</sup>
Meloxicam + Silymarin (n =40)	4.64 ± 0.22	3.92 ± 0.07* <sup>a</sup>	66.30 ± 3.54	40.7 ± 1.77* <sup>a</sup>

Values are presented as mean ± SEM; n = number of patients; \* significantly different compared with pre-treatment value within the same group ( $P<0.05$ ); data with non-identical superscripts (a, b, c) among different groups are considered significantly different ( $P<0.05$ ).

Table 2. Effects of silymarin on the serum levels of the activities of the liver enzymes, Alkaline phosphatase (ALP), Glutamate-oxaloacetate aminotransferase (GOT) and Glutamate-pyruvate aminotransferase in OA patients treated with piroxicam or meloxicam.

Treatment group	ALP (U/l)		GOT (U/l)		GPT (U/l)	
	Pre-treatment	post-treatment	Pre-treatment	post-treatment	Pre-treatment	post-treatment
Silymarin (n =20)	58.8 ± 4.6	43.9 ± 2.9* <sup>a</sup>	6.8 ± 0.4	5.8 ± 0.3* <sup>a</sup>	6.5 ± 0.6	5.2 ± 0.3* <sup>a</sup>
Piroxicam (n =35)	61.5 ± 6.9	69.7 ± 4.9* <sup>b</sup>	8.7 ± 0.6	9.2 ± 0.7 <sup>b</sup>	6.7 ± 0.5	10.6 ± 0.8* <sup>b</sup>
Piroxicam + Silymarin (n =40)	55.1 ± 3.2	43.9 ± 2.5* <sup>a</sup>	9.8 ± 0.1	7.0 ± 0.5* <sup>c</sup>	8.1 ± 0.6	5.5 ± 0.3* <sup>a</sup>
Meloxicam (n =32)	59.4 ± 2.6	69.4 ± 1.5* <sup>b</sup>	7.8 ± 0.6	8.8 ± 0.8 <sup>b</sup>	6.9 ± 0.3	6.1 ± 0.3 <sup>c</sup>
Meloxicam + Silymarin (n =40)	65.0 ± 2.8	49.2 ± 2.0* <sup>c</sup>	12.6 ± 0.9	6.3 ± 0.2* <sup>c</sup>	8.8 ± 0.7	5.7 ± 0.4* <sup>a</sup>

Values are presented as mean ± SEM; n = number of patients; \* significantly different compared with pre-treatment value within the same group ( $P<0.05$ ); data with non-identical superscripts (a, b, c) among different groups are considered significantly different ( $P<0.05$ ).

## Discussion :

Many chronically used drugs, including NSAIDs, produced different levels of renal and hepatic toxicities; <sup>(19)</sup> adverse renal effects have been reported in 5% of patients treated with NSAIDs, which mostly attributed to impairing the haemodynamic effects of prostaglandins in the renal system <sup>(20,21)</sup>. The results presented in table 1 indicated that although serum levels of urea and creatinine are within normal values in OA patients included in the study, treatment with silymarin alone for 8 weeks resulted in significant reduction in serum levels of both parameters compared to pre-treatment values, this might be attributed to the cytoprotective effect of silymarin against both the disease-and/or drugs-induced renal damage <sup>(11)</sup>. In the present study, the impact of treating OA patients with piroxicam or meloxicam on the renal function was clearly demonstrated, and found to be compatible with those reported by others <sup>(22,23)</sup>, where blockade of COX enzyme in the renal system impairs glomerular filtration which may be progressed to acute renal failure. <sup>(24,25)</sup> The results presented in this study (table 1) indicated that combination of silymarin with piroxicam or meloxicam resulted in improvement in renal function, revealed by significant reduction in serum urea and serum creatinine. Silymarin is known to be an effective free radical scavenger, causes significant reduction in lipid peroxidation, protecting and stabilizing cell membranes, <sup>(13, 14)</sup> it protects the renal system against drug-induced damage in many animal models, an effect attributed to its antioxidant and cytoprotective activities, <sup>(26,27)</sup> and the results reported in the present study can be explained on the same bases. Liver toxicity is one of the most widespread problems, both in the development of drugs and in their therapeutic applications. Most NSAIDs that produce overt hepatic injury (with jaundice) in low incidence lead to greater occurrence of mild hepatic enzyme abnormalities (GOT and GPT), which is reported in 5-15% of patients taking NSAIDs. <sup>(28)</sup> Confusion should be expected because liver function may be adversely affected by disease process which mimics drug injury. <sup>(29)</sup> In the present study (table 2), activities of hepatic enzymes in the serum were significantly elevated in OA patients treated with piroxicam or meloxicam, which might be attributed to the oxidative burden of drug metabolism in the liver. Combination of those NSAIDs with silymarin, or using silymarin alone in OA patients resulted in significant reduction in the activities of liver enzymes in the serum after 8 weeks. The hepatoprotective activity of silymarin has been reported in many experimental and clinical studies. In a rodent model, silymarin reduces or prevents liver

toxicity caused by the oxidative damage of many drugs used in the treatment of rheumatoid arthritis (RA), like chloroquine and acetaminophen; or against liver toxicity that experimentally induced by carbon tetrachloride or *amanita phalloid* toxin <sup>(30,31)</sup>. Silymarin was also found to be protective against ischemic liver injury and experimental model of inflammatory liver disease. <sup>(32)</sup> In the clinical practice, treatment of 20 patients having chronic active hepatitis with 240mg/day of silybinin-phosphatidyl choline complex for 7days significantly lowers serum liver enzymes (GOT, GPT, ALP) and total bilirubin. <sup>(33)</sup> Moreover, in 36 patients presented with alcoholic liver disease, treatment with 420mg/day of silymarin resulted in normalization of serum liver enzymes (GOT, GPT) activities, total bilirubin and an improvement in the histological profile of liver biopsies after six months of treatment. In addition, procollagen III peptides (a marker of acute fibrosis) were found to be significantly decreased during the treatment. <sup>(34)</sup> All these effects are consistent with the well defined antioxidant, cytoprotective and regenerative properties of silymarin, especially in the hepatic system. Accordingly, the results of the present study can be explained in this respect. In conclusion, silymarin when co-administered with the NSAIDs (piroxicam or meloxicam) decreases their renal and hepatic toxicities in OA patients.

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