

## Dose-dependent Anti-inflammatory Effect of Silymarin in Experimental Animal Model of Acute Inflammation

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### Abstract

Silymarin, a flavolignans from seeds of 'milk thistle' "*Silybum marianum*" has been widely used from ancient times because of its excellent hepatoprotective action. It has been used clinically to treat liver disorders including acute and chronic viral hepatitis, toxin/drug-induced hepatitis and cirrhosis and alcoholic liver disease. The efficacy and dose-response effect of silymarin (125, 250 and 500 mg/kg) were assessed using egg albumin-induced paw edema in rats as a model of acute inflammation. In this model, 56 rats were used and allocated into 7 subgroups each containing 8 rats. All treatments were given intraperitoneally 30 minutes before induction of inflammation by egg albumin and then the increase in paw edema was measured 1h, 2h and 3h after induction of inflammation by using the vernier caliper. The results indicated that silymarin, at doses range used, significantly lowered paw edema ( $P < 0.05$ ) an effect comparable to that produced by the reference drugs, acetyl salicylic acid, meloxicam and dexamethazone. Paw edema suppressive effect of silymarin 250 and 500 mg/kg was comparable and both of them were significantly different from that of silymarin 125 mg/kg ( $P < 0.05$ ). Therefore, silymarin exert an important anti-inflammatory activity in animal model of acute inflammation, which was significantly increased as the dose increased up to 250 mg/kg.

**Key words:** Silymarin, acute inflammation, dose-response

### الخلاصة

لقد استخدم الإنسان ومنذ القدم مادة السليمارين المستخلصة من بذور نبات الكعوب بسبب تأثيرها في حماية الكبد. ولقد استخدمت سريريا في الوقت الحاضر لمعالجة أمراض الكبد الحادة والمزمنة والناشئة عن الفيروسات أو التعرض للسموم والكحول. تم في هذه الدراسة تقييم التأثير المضاد للالتهاب لجرع مختلفة من مادة السليمارين (125، 250، 500 ملغم/كغم) في نموذج الالتهاب الحاد المستحدث بواسطة الألبومين في قدم الجرذان. تم استخدام 56 جرذا أبيض قسمت الى 7 مجموعات كل مجموعة ضمت 8 جرذان. تم علاج الجرذان بجرع مختلفة من السليمارين عن طريق الزرق بالبريتون قبل 30 دقيقة من استحداث الالتهاب بزرق زلال البيض في قدم الجرذان، وتم قياس مستوى تكوين الوذمة بعد ساعة واحدة، ساعتين وثلاث ساعات بعد استحداث الوذمة باستخدام الفيرنية. أظهرت النتائج بأن للسليمارين فعالية مضادة للالتهاب تعتمد على الجرعة مقارنة بالمركبات القياسية المستخدمة للمقارنة (حامض أسيتيل ساليسيليك، ديكساميثازون والميلوكسيكام). ومن خلال النتائج التي تم التوصل إليها يمكن الاستنتاج بأن للسليمارين تأثير مضاد للالتهاب في النماذج المختبرية للالتهابات الحادة والذي يزداد بزيادة الجرعة حتى 250 ملغم/كغم.

### Introduction

Inflammation is an important physiological reaction, which occurs in response to a wide variety of injurious agents (bacterial infection or physical trauma) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair (1). It requires the participation of various cell types expressing and reacting to diverse mediators along a very precise sequence (2). The inflammatory response is often initiated by the activation of resident macrophages through pattern-recognition receptors; this triggers the sequential release of pro-inflammatory mediators such as eicosanoids, cytokines, chemokines, and protease, which drive leukocyte recruitment and activation (3). Resolution of inflammation (anti-inflammatory response) is an active process controlled by

endogenous mediators that suppress pro-inflammatory gene expression and cell trafficking, induce inflammatory cell apoptosis and phagocytosis. An optional balance between pro- and anti-inflammatory responses is required to prevent the highly detrimental effect of extensive, prolonged or unregulated inflammation (3). Silymarin, the seed extract of milk thistle (*Silybum marianum*), is an ancient herbal remedy used to treat a range of liver and gallbladder disorders, including hepatitis, cirrhosis, and as a hepatoprotectant against poisoning from wild mushroom, alcohol, chemical, and environmental toxins (4). Milk thistle is one of the best-studied medicinal plants for the treatment of liver disease (4-7).

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Most of these effects have been attributed to direct and/or indirect antioxidant capacity of silymarin, such as being a scavenger of ROS, phenylglyoxylic ketyl radicals and a chain breaking antioxidant (8). The present study was designed to evaluate the efficacy and dose response effect of silymarin in experimental animal model of acute inflammation.

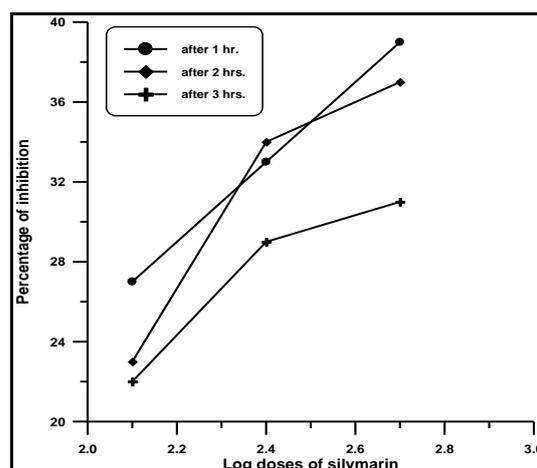
## Materials and methods

Silymarin powder used in the present was obtained as standardized pure extract from Luna Company (Egypt) and was dissolved in 98% dimethyl sulfoxide solution to produce stock solution with concentration of 250 mg/ml, from which different concentrations were obtained by dilution. Dimethyl sulfoxide and diethyl ether solutions were obtained from Merck Company (Germany), dexamethazone (American reagent, inc. USA), acetyl salicylic acid (Sanofi, France) and hand caliper (Germany). In the present study, 56 Sprague Dawley rats of both sexes (180-220 g) were used and allocated into 7 subgroups, each containing 8 rats. These groups represent control, standard and test groups. Silymarin was tested for its ability to suppress acute inflammation using fresh egg albumin-induced edema in rats as a model according to the technique established by Winter *et al* (9). Rats were fasted overnight, and deprived of water during the experiment to ensure uniform hydration and to minimize variability in edematous response (10). The rats were separated into 7 groups (8 rats each); control group treated with dimethyl sulfoxide 2 ml/kg; the three standard groups treated with acetyl salicylic acid 100 mg/kg, meloxicam 10 mg/kg and dexamethazone 1 mg/kg respectively; while the three test groups treated with silymarin (125, 250 and 500 mg/kg, respectively). All drugs were administered intraperitoneally and 30 minutes post-treatment, inflammation was induced by injecting 0.1 ml of fresh egg albumin (phlogistic agent) (11,12) into the sub planter surface of the right hind paw. The increase in paw edema, as a result of inflammation, was measured using vernier caliper before and 1 hr, 2 hr and 3 hr after induction of inflammation. The difference in paw thickness before and after induction of inflammation was calculated and presented as mean increase in paw thickness (mm). The ability of anti-inflammatory drugs to suppress paw inflammation was expressed as percentage of inhibition of paw edema (13). All results were expressed as mean  $\pm$  SEM. The significance of difference between the control and treated groups were determined using one-way

analysis of variance (ANOVA), followed by Student's *t*-test. *P*-values < 0.05 were considered significant.

## Results

The anti-inflammatory effect of silymarin on acute inflammatory model was shown in table 1 and figure 1. Silymarin (125, 250 and 500 mg/kg), acetyl salicylic acid, meloxicam and dexamethazone significantly reduced egg albumin-induced paw edema ( $P < 0.05$ ) compared with control group after 1 hr, 2 hr and 3r h from induction of inflammation. There is significant difference between silymarin (125 mg/kg) group and all other treatment groups ( $P < 0.05$ ) along three hours of assessment, while no significant difference exists between silymarin doses 250 and 500 mg/kg, and with dexamethazone and meloxicam groups at the second and third hour of assessment. Silymarin (250 and 500 mg/kg) produced an effect which is significantly different from that of acetyl salicylic acid at the second and third hour of assessment ( $P < 0.05$ ) (except for silymarin 500 mg/kg group which produces non significant effect at the second hour). The dose-response effect of silymarin on acute inflammation was illustrated in figure 1. The suppressive effect of silymarin on paw edema was significantly increased ( $P < 0.05$ ) as the dose doubled from 125 to 250 mg/kg. However, further increase in the dose up to 500 mg/kg did not show significant increase in the anti-inflammatory activity (except after the first hour, where silymarin 500 mg/kg significantly differs from silymarin 250 mg/kg).



**Figure (1): Dose-response effect of different doses of silymarin on egg albumin-induced acute inflammation in rats.**

**Table 1: Effect of different doses of silymarin on egg albumin-induced acute inflammation in rats.**

Treatment Groups	Mean increase in paw thickness (mm)			% of inhibition		
	1 h	2 h	3 h	1 h	2 h	3 h
Dimethyl sulfoxide 2 ml/kg	3.06 ± 0.06	2.29 ± 0.05	1.76 ± 0.06	–	–	–
Acetyl salicylic acid 100mg/kg	1.91 ± 0.06 <sup>*a</sup>	1.35 ± 0.04 <sup>*a</sup>	1.05 ± 0.04 <sup>*a</sup>	37	41	40
Meloxicam 10mg/kg	1.99 ± 0.05 <sup>*a</sup>	1.49 ± 0.05 <sup>*b</sup>	1.15 ± 0.05 <sup>*a</sup>	35	35	35
Dexamethazone 1mg/kg	1.78 ± 0.06 <sup>*b</sup>	1.37 ± 0.06 <sup>*a</sup>	1.10 ± 0.05 <sup>*a</sup>	42	40	37
Silymarin 125 mg/kg	2.24 ± 0.06 <sup>*c</sup>	1.76 ± 0.05 <sup>*c</sup>	1.38 ± 0.06 <sup>*b</sup>	27	23	22
Silymarin 250 mg/kg	2.05 ± 0.07 <sup>*a</sup>	1.51 ± 0.06 <sup>*b</sup>	1.25 ± 0.06 <sup>*c</sup>	33	34	29
Silymarin 500 mg/kg	1.85 ± 0.05 <sup>*b</sup>	1.45 ± 0.04 <sup>*b</sup>	1.21 ± 0.04 <sup>*c</sup>	39	37	31

Data were expressed as mean ± SEM; number of animals = 8 in each group; \* $P < 0.05$  with respect to control group; values with non-identical superscripts (a, b, c) among different groups are considered significantly different ( $P < 0.05$ ).

## Discussion

The inflammatory response is a physiological characteristic of vascularized tissues<sup>(14)</sup>. Exudation, which is a consequence of increased vascular permeability, is considered as a major feature of acute inflammation<sup>(15)</sup>. Egg albumin-induced paw edema in rats is an *in vivo* model of inflammation used to screen agents for anti-inflammatory effect<sup>(16)</sup>. The characteristic swelling of the paw is due to edema formation. Inhibition of increased vascular permeability and hence the attendant edema modulate the extent and magnitude of the inflammatory reaction. The paw edema that induced by injection of egg albumin is peaked after 1 h and then progressively decreased with time. Many chemical mediators like histamine, 5-HT, kinins and prostanoids mediate acute inflammation induced by phlogistic agents including egg albumin<sup>(17)</sup>. In accordance with Marsha-Lyn *et al*<sup>(18)</sup>, inflammation occurs through three distinct phases: an initial phase mediated by histamine and 5-HT (up to 2 hour); an intermediate phase involving the activity of bradykinin and a third (late) phase with prostanoid synthesis by COX<sup>(19)</sup>. The anti-inflammatory activity of silymarin extract was evaluated by egg albumin-induced paw edema using vernier caliper method and the results were shown in table 1 and figure 1. Three different doses (125, 250 and 500 mg/kg) of silymarin were evaluated for the aim of finding dose-response relationship; the results clearly indicated the significant anti-inflammatory activity of this flavonoid within the dose range utilized, compared to the standard anti-inflammatory agents used in this respect. It efficiently suppressed early,

intermediate and late phases of acute inflammation as illustrated in table(1). However, the effect of silymarin on early and intermediate phases was better than that on the late phase as shown in figure (1). The anti-inflammatory effect of silymarin, especially at the doses 250 and 500 mg/kg was comparable to that of standard drugs and there was no significant difference between them. Additionally, silymarin shows a dose-dependent effect up to 250 mg/kg, and further increase of the dose was not associated with further increase in activity. The *in vivo* anti-inflammatory activity of silymarin was tested in different experimental models of inflammation, and the results suggested that an important anti-inflammatory action was achieved by inhibition of neutrophils migration into the inflamed site which lead to the release of ROS, RNS and proteolytic enzymes resulting in microvascular endothelial injury, increase endothelial barrier permeability and edema<sup>(20)</sup>. Silymarin at doses range 25, 50 and 100 mg/kg, when administered orally, significantly reduced papaya latex-induced paw inflammation. However, it was not effective against carrageenan-induced inflammation; it also reduced experimentally-induced ear edema in mice (36.84%) but the reduction was statistically not significant<sup>(21)</sup>. Silymarin has many biological activities as antioxidant, anti-inflammatory, cytoprotective and anticancer effects, it scavenges free radicals, increases cellular GSH content and induces superoxide dismutase (SOD) activity and causes a significant reduction in lipid peroxidation and consequently protecting and stabilizing cell membranes<sup>(22, 23)</sup>. The cell membrane stabilizing effect of silymarin on

mast cell<sup>(20)</sup> may explain its effect on the initial phase of acute inflammation model mediated by histamine and 5-HT. It may suppress mast cell degranulation and inhibits the release of these mediators that initiate early phase of paw inflammation. Silymarin blocked TNF- $\alpha$ -induced activation of NF- $\kappa$ B which regulates the expression of various genes involved in inflammation, cytoprotection and carcinogenesis in a dose and time dependent manner<sup>(24)</sup>, and it is completely blocked the mRNA expression of IL-1 $\beta$  and COX-2 in lipopolysaccharide (LPS) stimulated RAW 264.7 cells<sup>(25)</sup>. Additionally, the strong inhibition of leukotriene B4 (LTB4) formation by silybinin, which is the major component extracted from milk thistle and considered to be most biologically active in terms of its antioxidant and anti-inflammatory properties<sup>(26)</sup>, was confirmed in experiments with phagocytic cells isolated from human liver, with a significant inhibition of 5-LOX achieved *in vivo*<sup>(27)</sup>. Inhibition of PG synthesis and COX expression by silymarin may explain its effect on the third phase of inflammatory reaction. It is now clear that silymarin suppresses early, intermediate and late phase of acute inflammatory model through interference with the initiation and propagation phases of acute inflammation. However, there is limited data about the role of silymarin in the resolution phase of inflammation, which is recently considered as an important target for drugs used in inflammatory diseases. Leukocytes migration contributes to initiation and propagation of acute inflammation which was significantly inhibited by silymarin. Propagation phase also mediated by a diverse number of inflammatory mediators whose release, activity and genetic expression was significantly inhibited by silymarin<sup>(25)</sup>. In comparison with silymarin, NSAIDs inhibit propagation phase of inflammation through inhibition of PG synthesis, but they have negative effect on the resolution phase of inflammation (except acetyl salicylic acid) through inhibition of 15d-PGJ2 synthesis, which is important pre-resolving anti-inflammatory mediator<sup>(28)</sup>. On the other hand, steroids are considered as a gold standard of anti-inflammatory drugs where they inhibit all phases of acute inflammation. They inhibit initiation and propagation phases through suppression of leukocytes migration and inflammatory mediators genetic expression while enhance resolution phase of inflammation through augmentation of macrophages capacity for phagocytosis of apoptotic cells<sup>(29)</sup>. In conclusion, silymarin in a dose dependent pattern was effective in

decreasing acute inflammatory reactions in experimental animal models. The anti-inflammatory activity of silymarin increased up to 250 mg/kg and further increase of the dose will not result in further increase in activity.

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