

The Efficacy of Topically Applied Silymarin in the Treatment of Herpes Labialis Ulcers

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Abstract :

Herpes labialis is an infection caused by the herpes simplex virus, characterized by an eruption of small and usually painful blisters on the skin of the lips, mouth, gums, or the skin around the mouth. Although there is no successful treatment available, the local use of compounds with effective anti-inflammatory and cytoprotective effects may be of value in this respect. This project was designed to evaluate clinically the local use of silymarin, a group of flavonoids with powerful antioxidant, anti-inflammatory and cytoprotective activity, in the treatment of herpes simplex ulcer. Fifty three patients with herpes labialis ulcers (HLU) were enrolled in this randomized, single blinded, placebo controlled clinical study, and they were allocated into 4 groups, treated with 1%, 3% and 5% silymarin paste and placebo formula respectively. Patient's responses to treatment were followed by clinical evaluation of healing time, size of the ulcers and pain sensation, in addition to evaluating biochemical and immunological markers of the oxidative stress and inflammatory response. HLU patients showed dose dependent improvement in the healing time, pain score and size of ulcer as a result of treatment with various concentrations (1%, 3% and 5%) of silymarin paste, associated with improvement in the oxidative stress state and immunological parameters. In conclusion, silymarin can be used locally as paste formula for the treatment of HLU, an effect which may be attributed to its antioxidant, anti-inflammatory and cytoprotective properties.

Keywords: Herpes labialis, ulcers, silymarin

الخلاصة

تعتبر تقرحات الفم التي يسببها فيروس الحلأ البسيط من الأمراض الالتهابية المؤلمة الشائعة التي تصيب الأغشية المبطنة للفم. وبالرغم من عدم وجود علاج فعال لمثل هذه الحالات، فإن استخدام مركبات لها فعالية مضادة للالتهاب وحماية خلوية فعالة قد يكون ذا فائدة في هذا المجال. تم تصميم هذه الدراسة لتقييم الاستخدام الموضعي لمادة السيليمارين في علاج تقرحات الفم المتكررة. أجريت هذه الدراسة على 53 مريضا بتقرحات الفم التي يسببها فيروس الحلأ البسيط، حيث تم تقسيمهم إلى أربعة مجموعات تم علاجها بـ 1% أو 3% أو 5% سيليمارين بشكل معجون موضعي تم تحضيره خصيصا لهذا الغرض أو مستحضر تهدئة على التوالي. تمت متابعة استجابة المرضى للعلاج من خلال حساب الفترة الزمنية اللازمة للالتام القرحة ومعدل التغيير في مساحتها وكذلك مستوى تسكين الألم الناشيء عنها، بالإضافة إلى تقييم بايوكيمياوي ومناعي لبعض المعايير الخاصة بالالتهاب وفرط الأوكسدة مثل الأنترلوكين، الكلوبينات المناعية، المالونداي الديهايد والكلوتاثيون. أظهرت نتائج الدراسة تحسنا ملحوظا في استجابة المرضى للعلاج من خلال انخفاض الفترة الزمنية اللازمة للالتام القرحة وانخفاض مستوى الشعور بالألام الناشئة عنها، إضافة إلى تحسن واضح في معايير فرط الأوكسدة والمعايير المناعية الأخرى مقارنة باستخدام مستحضر التهدة. وعليه يمكن الاستنتاج بإمكانية استخدام السيليمارين كمستحضر موضعي في علاج تقرحات الفم الناشئة عن الإصابة بفيروس الحلأ البسيط.

Introduction :

Herpes simplex virus (HSV) affects more than one third of the world's population, and is responsible for a wide array of human diseases, with effects ranging from discomfort to death.⁽¹⁾ HSV actually belongs to a family of eight related viruses, including HSV1, HSV2, varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and cytomegalovirus (CMV); all of them are double-stranded DNA viruses, which permanently infect their target cells.⁽²⁾ HSV produces infections ranging from cold sores to severe genital lesions; and both the initial episode and recurrent disease can cause devastating problems.⁽³⁾ It affects the skin, mucous membranes, and less frequently the

esophagus and brain. Skin infections are usually located in the orolabial, genital, or anorectal area. Of the two serotypes, HSV-1 infection is primarily oropharyngeal and HSV-2 infection is primarily genital.⁽⁴⁾ Among the many clinical expressions of HSV-1 infections, the easiest to recognize is the typical cold sore or fever blister.⁽⁵⁾ Many patients have pain, burning and considerable anxiety which justify the use of systemic drug therapy. Many medications are available for treatment of herpes, including foscarnet sodium, gancyclovir, but the three main antiviral drugs used are acyclovir, valacyclovir and famciclovir. There are a number of alternative treatments that have been

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anecdotally reported to be effective in treating HSV infection; for example, L-lysine, naturally occurring in many foods, can be used effectively in this respect.⁽⁶⁾ Herbs that may be helpful in HSV infection include lemon balm (*Melissa officinalis*) which has antiviral activity.⁽⁷⁾ Commercial silymarin is a standardized preparation extracted from the fruits (seeds) of *Silybum marianum*, consists of three major flavolignans; silybinin, silychristin and silydianin, of them Silybinin is the most biologically active.⁽⁸⁾ Silymarin has cytoprotective, antioxidant and anti-inflammatory activities,^(9, 10, 11) thus it may be helpful in many inflammatory disorders like ulcers associated with HSV infection. It is regarded as being non-toxic with high margin of safety and no serious adverse reactions have been reported due to its clinical use. This study was designed to evaluate the clinical utility of using silymarin as locally applied pharmaceutical dosage form in the treatment of HLU.

Patients and Methods :

Trial Design: Fifty three patients with HLU (age range 5-64 ± 12 .23 years, 30 females and 23 males) were included in a randomized, placebo controlled, single blinded clinical trial conducted in the Department of Oral Medicine, College of Dentistry, University of Baghdad during the period Jan-Dec/2004; patients selection was performed according to the following criteria: The patients should have a history of recurrent ulceration due to herpes simplex, female patients should not be pregnant, they should not suffer from any chronic debilitating disease and not receive any antibiotics or steroids medication. All patients were informed about the nature of the study and their signed consent was obtained. After careful diagnosis and characterization of HLU patients, they are randomized and treated as follow: Group A includes 14 patients treated with 1% silymarin paste 4 times daily; group B includes 14 patients treated with 3% silymarin paste 4 times daily; group C includes 15 patients treated with 5% silymarin paste 4 times daily; group D includes 10 patients treated with placebo formula containing all the ingredients of the oral paste formula except the active constituent (silymarin) 4 times daily. Oral paste formula of silymarin for topical application was prepared according to B.P. (1997),¹² in different strengths (1%, 3%, and 5% Silymarin w/w). Placebo formula which contains all the ingredients except the active constituent (Silymarin) was prepared in the same way. Each patient was followed regularly by daily inspection during the period of application of the tested medication to the ulcer to determine the degree of pain, size of ulcer, and the end point time of healing process.

Analysis of Immunological and Biochemical parameters

Five ml of venous blood was drawn from 6 patients belongs to group C and 6 patients belongs to group D before starting treatment and after 3 days of treatment. After preparation of serum, the levels of Interleukin-1 α , complement proteins C₃ and C₄, the immunoglobulin IgG, IgA, and IgM and the oxidative stress parameters malondialdehyde (MDA) and glutathione (GSH) in the serum were analyzed according to standard procedures.^(13, 14, 15, 16) Statistical evaluation of data was performed utilizing paired Student's *t*-test, while ANOVA was performed to compare between different groups. Differences were considered significant at *P* values <0.05.

Results :

Figure 1 clearly demonstrated that greater percentage of HSU patients showed faster and complete ulcer healing due to treatment with silymarin within 3-6 days, which was found significantly different with respect to placebo-treated group (11-15) days. 5% silymarin formula produces significantly higher rate of ulcer healing compared to 1% and 3% formulas. Figure 2 showed that at day 2 significant reduction in mean ulcer size (*P*<0.05) was produced by both 5% and 3% silymarin compared with 1% formula. All silymarin formulas produced significant reduction in mean ulcer size compared to placebo within 5 days of the follow up period. Figure 3 showed that treatment with silymarin paste (1%, 3% and 5%) resulted in highest number of patients with complete pain relief, as measured by visual analogue scale (VAS), which was significantly different compared to placebo treated group (*P*<0.05). However, treatment with 5% silymarin paste resulted in significantly higher percentage of patients with complete pain relief compared with 1% and 3% silymarin formulas during the days 1 and 2 after starting treatment; while during the days 3 and 4 no significant differences were observed in this respect. Figure 4 showed that mean pain score in all HLU patients were gradually decreased over 5 days of the study, with 5% silymarin treated group showing a mean pain score of 1, 0.5 and 0 during the days 1, 2 and 3 respectively, a result which is significantly lower than those observed in other groups (*P*<0.05). However, even when 1% and 3% silymarin treated groups reported significantly lower mean pain score compared to placebo, they did not show significant differences between them. Concerning the effects of treatment with 5% silymarin on the immunological and biochemical markers in the serum of patients, table 1 indicated that treatment with 5% silymarin paste for 4 -5 days resulted in significant reduction in serum level

of IL-1 α (26%, $P<0.05$) compared to pre-treatment level, while in placebo-treated patients IL-1 α show significant elevation (20%, $P<0.05$) compared to pre-treatment levels. When the two types of treatment (silymarin vs placebo) were compared, significantly lower values for serum IL-1 α were observed after 4 -5 days of treatment in the silymarin-treated group compared to placebo-treated one (35%, $P<0.05$). Table 1 also showed that treatment with 5% silymarin paste for 4-5 days produced significant reduction in serum levels of the complement proteins C3 and C4 (13% and 44% respectively) compared to the pre-treatment levels. Placebo formula did not produce any significant changes in this respect. When the two methods of treatment compared (silymarin vs placebo), no significant differences reported in C3 levels ($P<0.05$), while C4 levels showed significantly lower values due to silymarin treatment (43%) compared to placebo treatment. Table 2 demonstrated that treatment of HLU patients with 5% silymarin paste for 4 -5 days resulted in significant reduction in the serum levels of the immunoglobulins IgG, IgM and IgA (4%, 22% and 36% respectively) compared to pre-treatment levels, while placebo treatment did not alter Ig's levels during the same period of treatment. When the two methods of treatment were compared, silymarin paste showed significant lowering effect in Ig's levels compared to placebo. Table 3 demonstrated that treatment of HLU patients with 5% silymarin paste for 4 -5 days resulted in a significant reduction in the serum MDA level compared to pre-treatment level (49%, $P<0.05$). Meanwhile, placebo-treated group did not show such an effect. When the two types of treatment (silymarin vs placebo) were compared, significantly lower value of serum MDA level was observed (54%, $P<0.05$) due to silymarin treatment compared with placebo. Concerning GSH levels, table 3 showed that treatment with 5% silymarin paste for 4 -5 days resulted in significant elevation in serum GSH level (48%, $P<0.05$) compared to pre-treatment value. Meanwhile; treatment with placebo formula did not show such an effect, and GSH levels continuously declined. When the two types of treatment (silymarin vs placebo) were compared, significantly higher value of serum GSH was observed in silymarin treated group (19.8%, $P>0.05$) compared with placebo-treated group.

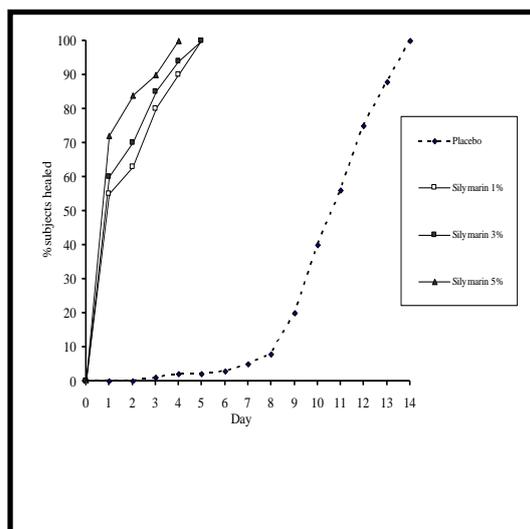


Figure 1. Effects of treatment with silymarin paste on ulcer healing time in HLU patients. All results are significantly different ($P<0.05$) compared to placebo within 3-5 days.

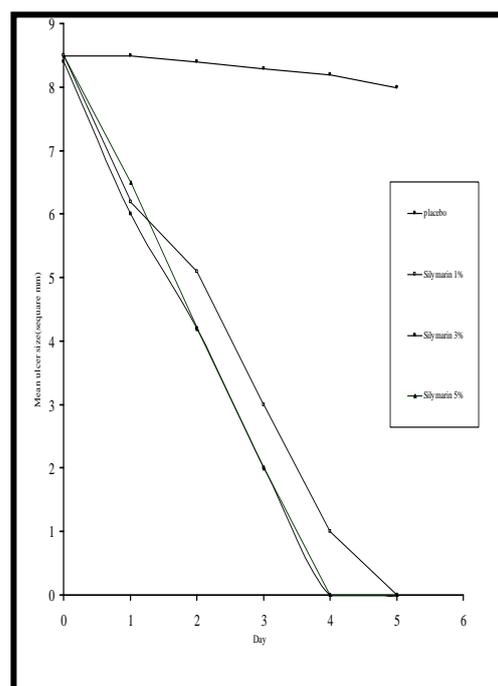


Figure 2. Effects of treatment with silymarin paste on the changes in ulcer size in HLU patients. All results are significantly different ($P<0.05$) compared to placebo within 5 days.

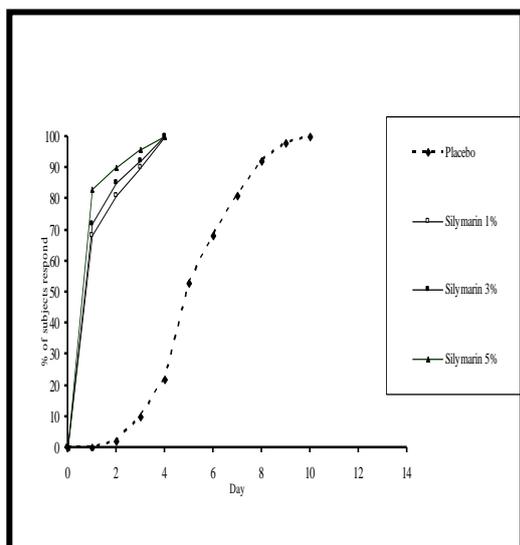


Figure 3. Effects of treatment with silymarin paste on complete pain relief in HLU patients. All results are significantly different ($P<0.05$) compared to placebo within 3 days.

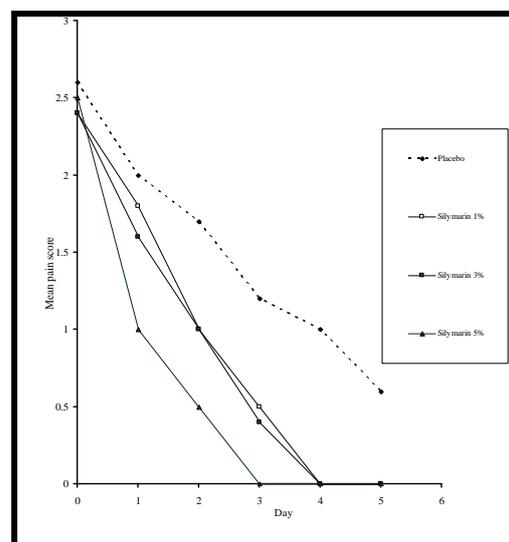


Figure 4. Effects of treatment with silymarin paste on the pain score in HLU patients. All results are significantly different ($P<0.05$) compared to placebo within 3 days.

Table 1. Effects of silymarin 5% paste on serum levels of interleukin 1- α (IL-1 α) and the complement proteins (C3 and C4) in HLU patients.

Type of treatment	IL-1 α pg/ml		C3 mg/dl		C4 mg/dl	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Silymarin 5% n = 6	486.5 \pm 19.24	360.8 \pm 41.34* ^a	173.6 \pm 11.2	150.16 \pm 12.6* ^a	33.05 \pm 1.37	18.4 \pm 1.58 * ^a
Placebo n = 6	460 \pm 55.68	550 \pm 36.05* ^b	171.23 \pm 6.8	171.23 \pm 6.85 ^a	32.16 \pm 0.75	32.16 \pm 0.75 ^b

Values are presented as mean \pm SD; n = number of subjects; * significantly different with respect to pre-treatment period ($P<0.05$); values with non-identical superscripts were considered significantly different ($P<0.05$) among different groups.

Table 2. Effects of silymarin 5% paste on serum immunoglobulins levels in HLU patients.

Type of treatment	Silymarin 5% n = 6	Placebo n = 6	
IgG mg/dl	Pre-treatment	1409.45 \pm 116.39	1475.56 \pm 58.2
	Post-treatment	1362.20 \pm 117.7 * ^a	1475.56 \pm 58.2 ^a
IgM mg/dl	Pre-treatment	191.60 \pm 33.94	207.46 \pm 21.95
	Post-treatment	149.56 \pm 37.17 * ^a	207.46 \pm 21.95 ^a
IgA mg/dl	Pre-treatment	199.45 \pm 11.98	204.46 \pm 5.94
	Post-treatment	127.88 \pm 7.144 * ^a	204.46 \pm 5.94 ^b

Values are presented as mean \pm SD; n = number of subjects; * significantly different with respect to pre-treatment period ($P<0.05$); values with non-identical superscripts were considered significantly different ($P<0.05$) among different groups.

Table 3. Effects of silymarin 5% paste on serum malondialdehyde (MDA) and glutathione (GSH) contents in HLU patients.

Type of treatment	MDA ($\mu\text{mol/l}$)		GSH ($\mu\text{mol/l}$)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Silymarin 5% n=6	3.04 \pm 0.32	1.56 \pm 0.09 * ^a	13.35 \pm 1.7	19.72 \pm 3.39 * ^a
Placebo n=6	3.08 \pm 0.31	3.38 \pm 0.2 * ^b	10.04 \pm 1.52	6.617 \pm 0.73 * ^b

Values are presented as mean \pm SD; n = number of subjects; * significantly different with respect to pre-treatment period ($P < 0.05$); values with non-identical superscripts were considered significantly different ($P < 0.05$) among

Discussion:

Herpes virus, which consist of a single double-stranded DNA molecules enclosed in a viral envelope, produce ulcers as a result of triggering an array of host responses that gives rise to enhanced inflammatory mediators and cytokine responses in macrophages and other host cells.⁽¹⁷⁾ There is no curative therapy, but antiviral medications given by mouth may shorten the course of the symptoms and decrease pain. The flavonoids (including those present in silymarin) have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities.^(18, 19) Topical use of silymarin paste demonstrates improved efficacy in the treatment of HSU compared to placebo, as measured by the primary (objective) outcome of complete ulcer healing and, albeit less robustly, the secondary (subjective) outcome of complete pain relief. The anti-inflammatory activity of silymarin components (silybinin) was assessed in human PMNs *in vitro*; where the chemotactic and phagocytic activities of these PMNs were not modified by silybinin at concentrations of 0.5-25 $\mu\text{g/ml}$.⁽²⁰⁾ Reactive oxygen species (ROS) released by neutrophils and other phagocytes have been increasingly implicated in inflammatory immune disorders.^(21, 22) Flavonoids, including silymarin, could profoundly impair the production of ROS by inflammatory cells; this may be accomplished by interference with NADPH-oxidase, a powerful oxidant-producing enzyme localized on the surface of neutrophil's plasma membrane.⁽²³⁾ They could also inhibit neutrophils myeloperoxidase (MPO), a source of reactive chlorinated intermediates.⁽²⁴⁾ The data presented in this study clearly demonstrated that mean ulcer size in the placebo-treated group showed no significant differences during 0-5 days, compared to the gradual decrease reported in silymarin-treated

group during 1-4 days, this suggested that silymarin exerted a powerful effect on healing process, and initiates it early during the course of HLU. Cytokines played an important role in the immune response to HSV infections; in particular TNF- α , which is primarily produced by macrophages, and is known to be central for control of virus replication.^(25, 26) However, TNF- α and TNF- α -induced products are also involved in the immunopathology often associated with HSV infection.^(27, 28) Silymarin has anti-inflammatory and cytoprotective effects by suppression of NF- κB , the nuclear transcription factor, which regulates the expression of various genes involved in inflammation. It blocks TNF- α -induced activation of NF- κB in a dose and time dependent manner. This effect was mediated through inhibition of phosphorylation and degradation of Iota Kappa B alpha, an inhibitor of NF- κB .⁽²⁹⁾ Since replication of certain viruses is dependent on NF- κB ,⁽³⁰⁾ silymarin may interfere with viral replication. Mucsi and Pragai (1985) demonstrated the inhibitory effect of four flavonoids in human herpes simplex viruses type I, and there was a relationship between viral inhibition and the ability of the studied flavonoids to increase cyclic AMP in the HEP-2 cells and chicken embryo fibroblasts.⁽³¹⁾ Phospholipase A₂ (PLA₂) is likely an important intra- and extra-cellular mediator of pain mediators during inflammation.⁽³²⁾ Silymarin was found to be an effective inhibitor of PLA₂ from human sources; additionally, silybinin, silydianin and silychristin (constituents of silymarin) were found to effectively blocking the activity of lipoxygenase and prostaglandin synthesis *in vitro*.⁽³³⁾ These molecules are intimately involved in inflammation and allergy, as well as in many other physiologic and pathologic processes including pain. Silybinin inhibits synthesis of leukotriene B₄ (LTB₄) (IC₅₀ = 15

µmol/L) in isolated rat Kupffer cells; others showed that the three pharmacologically active components of silymarin, namely silybinin, silydianin and silychristin inhibit cyclooxygenase (COX) enzyme *in vitro*.⁽³³⁾ All the previous events indicated that silymarin may act as analgesic agent through these pathways, and this might explain the reduction in pain observed in the present study. According to the results presented in this study, serum levels of IL-1 α decreased significantly after treatment with silymarin paste compared to pre-treatment values, while placebo-treated group did not show such effect; this observation may give another indication that can be added to those deal with the inhibitory effects of silymarin on interleukin production with consequent anti-inflammatory activity. Antibodies to HSV are mostly of IgG type, although HSV-specific IgA is also detectable.^(34, 35) All the observations about the possible role of IgG, IgM, IgA and the complement protein components (especially C3) supported the concept of occurrence of immune complex vasculitis, which is found essential step in the pathogenesis of oral ulceration.⁽³⁶⁾ The results presented in this study concerning the changes in the levels of serum Immunoglobulins (IgA, IgG and IgM) are compatible with some of the previously indicated studies, where significant differences between pre- and post-treatment with topical silymarin paste were observed compared to placebo in HLU patients. Phagocytosis is an important physiological process accompanied by production of superoxide anion, and activated phagocytic cells such as monocytes, neutrophils and macrophages; this process is extremely important for their bactericidal functions.⁽³⁷⁾ While ROS generated by phagocytes play an important physiological function, they can also cause cellular damage. ROS along with other mediators generated by neutrophils and macrophages can promote inflammation and cause tissue damage.⁽²⁴⁾ The consequently resulted state of oxidative stress can produce damage to many biological molecules; where proteins and DNA are definite targets and predispose to cellular injury. Lipid peroxidation is often a secondary event consequent to primary cell damage induced by oxidative stress. In the present study, local treatment with silymarin paste produces inhibitory effects on lipid peroxidation, as revealed by significant decrease in serum MDA levels compared to placebo, which very well indicated the powerful antioxidative effects of silymarin. Silymarin prevents liver glutathione depletion and lipid peroxidation induced by an acute intoxication with ethanol in rats.⁽³⁸⁾ These effects supported the suggested action of silymarin as a cytoprotective agent. In the present study, silymarin produced significant

increase in serum glutathione (GSH) levels compared to placebo. Silymarin is known to induce superoxide dismutase enzyme (SOD) and glutathione biosynthesis; it brings GSH level back to normal after depletion; so it is not only prevent depletion of GSH, but increases the basal level of activity of detoxifying enzymes.⁽³⁹⁾ In conclusion, silymarin can be used topically for the treatment of HLU; its high efficacy and safety make it a good alternative for the available pharmacological agents in this respect.

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