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Anti-Inflammatory Effects of Silymarin Against Damages Caused by U.V. Irradiation

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Abstract: In this study, the protective traits of Silymarin as an herbal medicine were investigated. To this study, 24 albino guinea pigs that were in same age and sex, selected and divided to 4 groups, randomly. Dorsal region of body was shaved in 2×2 cm^2 dimension. First treatment group received 9 mg Silymarin with 20 µlacetone, topically and control group just received acetone. Second treatment group received 50 mg Silymarin, orally and control group received nothing. All groups were irradiated with UV 180 mj/cm^2 for 35 min/day and total time of irradiation was 10 day. The resultsofClinical and Pathological observations showed that Silymarin, especially in topical administration, have significant protective effects on UV lesions. This study showed that Silymarin anti-inflammatory effects are considerable in skin protection against UV radiation damages, especially when used topically.

Key words: Anti-Inflammatory · Silymarin · UV Irradiations · Skin

INTRODUCTION

Inflammation is the reaction of body tissues against Stimulation and injuries. Inflammation is an essential protective mechanism because has a chemotactic effect on immunoglobulin, complement and phagocytes and attract these immune factors to injured or microbial attacked tissues [1]. Many environmental factors cause to injuries and inflammation in the skin, especially sun light that is an important environmental factors in skindamages such as skin cancer [2]. The main elements of sunlight that have pathological effects on the human skin include UV and visible wave lengths between 290 to 700 nanometer. Also, wave lengths up to 700nm in infrared spectrum, primarily cause heat transmission and in the definite conditions, can increase harmful effects of UV and visible spectrum [3]. Today there are widespread tendency to use natural components to sunlight inflammatory and harmful effectssunlightprevention and there are many researches about skin protection with herbal compounds[4]. Silybiummarinum is one of this plants that belong to Astrasea or compozitea family. Among the known antioxidant compounds of this planet (Carotenoid, tocoferols, vitamin E, vitamin C and etc.) can be cited to large family of fenolic compounds. Silymarin is one of these compounds that can be found in all plant parts but has more gathering in fruit and seed [5]. Silymarin pure compound has 7-chromanol 3-methyl taxifulinstructure.A number of other flavnolignans are in the seeds. Studies have shown that these compounds protect different damages of various organs and cells [6]. Prevention of IL-10 using Silymarincan protect incidence of immune system disorders and immune suppression in the body of laboratory rats [2]. Anti-inflammatory effects of Silymarin have been approved on articular inflammations [7]. Also, Silymarin is shown to be used as a strong antioxidant compound with Stimulation and increased production of antioxidant substances in the

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cellsandstrengthening activities against cells free radicals [8]. In this study, anti-inflammatory effects of Silymarin have been studied against damages due to UV irradiation on the skin of albino guinea pigs. Also, changes in immune cellsbeen studied in inflammatory position due to UV-B irradiation to be specified the effect of topical and oral Silymarin on these cells.

MATERIALS AND METHODS

4 groups of six albino guinea pigs in same age (6 months) and sex were selected and in order to match with new environment were kept in standard environmental and nutritional conditions. Four groups studied in this experiment were:

First Treatment Group: In this group 9 mg per animal Silymarin, after solve in20 µl acetone, applied topically on a 2×2cm² of skin.

First Control Group: In this group, as a control group of treatment 1, only acetone applied topically on a 2×2cm² of skin.

Second Treatment Group: In this group Silymarin were fed orally to guinea pigs (50 mg per animal).

Second Control Group: This group, as a control group of second treatment group, didn't received Silymarin (neither orally and nor topically).

At the end of accordance period in fifteen day, dorsal part of animals was shaved with a razor in 2×2cm^2 dimensions. After each time of hair shaving, the animals were resting for around 48 hours for decreasing inflammation of the shaving. In seventeenth day, all animals in each group were exposed to radiation. In first group, Silymarin solution (Silymarin+acetone) applied locally with a swab on the shaved part of guinea pigs skin, 30 min before animal's placement in exposed with ultra violet radiation. After 30 minutes, guinea pigs were completely restricted in special cages and then for 35 minute were exposed to ultraviolet radiation due to ultraviolet light. The distance between light and animal skins was 20 cm. All the preparation was carried out in the first group, in the same order was used in second group. But instead of using Silymarin in this group, only acetone applied topically on the animal's skin, 30 minutes before radiation exposure. In third group, five days before UV exposure, silymrin was used for guinea pigs, orally. Thus, 12 days after beginning of the study, the calculated amounts of Silymarin mixed in 2 ml water and were fed to the animals by a dropper. After seventh day, similar to previous two groups, after restriction of guinea pigs in special cages, they were exposed to ultraviolet radiation UV-B for 35 minutes. In forth group, Silymarin didn't provide for guinea pigs, but this group was in similar environmental and nutritional conditions with other three groups and from seventeenth day was exposed to UV radiation for 35 minutes (Radiation dose in all groups was 180millijoul/cm^2).

It should be mentioned that in this study, guinea pigs hairs was shaved twice in any of 4 groups, Once a day on days 15 and 23. After each time of hair shaving, the animals were resting for around 48 hoursand then for 5 days were exposed to radiation.

At the End of twenty-third day, all of the guinea pigs after anesthesia with ketamine (25mg/pKg) and acepromazine (25ml/pKg) were euthanatized with location change of neck vertebrates. Sampling, in order to histopathological study, was made from places that guinea pigs hairs were shaved. Samples obtained from each group were placed separately in Containers containing formalin 10% and were transferred to the pathology laboratory. In laboratory, after primary preparations, several sections were prepared from each sample. After staining with hematoxylin-eosin color, slides were seen with light microscope for pathological evaluations.

RESULTS

At the end of this study, different areas of animal skin that exposed to UV-B radiation, studiedmicroscopically (Table 1-1):

DISCUSSION

Many medicinal effects are mentioned for Silymarin and recently have been consideredas anti-inflammatory effects. Various ways exists for evaluation of inflammation presence in damaged tissue that has been used in many studies. Katiyar *et al.* [9] studied anti-inflammatory effects assessment of Silymarinagainst the lesions caused by UV-B radiationon the hairless mice's skin. This study was conducted with interloukin 10 measurement (as an indicator for tissue inflammatory presence) by ELISA, both in dermis and epidermis. Statistical studies indicate the existence of 59% Interloukin 10 in dermis and 73% in epidermis ($P<0.001$). In this study, number of Interloukin 10 producer cells in the UV exposure skins increased to skins that weren't

Table 1-1: Results of pathological studies in guinea pigs skin at the end of study

Animal groups injuries	Treatment1: topically Silymarin+ acetone	Control1: Only acetone	Treatment2: orally Silymarin	Control2: only were kept in standard conditions
Epidermal hyperkeratosis	-	+++	+	+++
Lymphocyte infiltration into epidermis (exocytose)	-	+++	-	+++
Squamous cell proliferation (akantosis)	-	+++	-	+++
Lymphocyte infiltration to the sebaceous glands	-	++	-	++
Edema and dermal thick increase	+	+++	++	+++
Lymphocyte, plasma cell and eosinophil infiltration indermal area	+	+++	++	+++

exposed with UV. It should be mentioned that treatment with Silymarin significantly reducesinterloukin10 producer cells and interleukin production in the inflammed skin with UV that indicates decrease in leukocyte infiltration an inflammation.

Bradley *et al.* [10] reported anti-inflammatory effects of Silymarin, after two weeks of UV-B radiation, measured activity of Myeloperoxidase enzyme in dermis and epidermis of hairless mice. Treated group with topical Silymarinshoweda significant reduction in Myeloperoxidase activity (71 % and 50% in the epidermis and dermis, respectively). Myeloperoxidase is recognizing as a tissue infiltration indicator.

Meeran *et al.* [4] showed for first time that intra peritoneal injection of Silymarin in mice prevents from UV-B irradiation immune suppression that can cause skin cancer. This inhibition is due to increase in the rate of specific IL-12. In another study, Mukhtar [11] measured NO and H_2O_2 rate in damaged tissue and evaluated Silymarin antioxidant activity as part of the anti-inflammatory activity of this matter.

Zhao *et al.* [12] study determined that Silymarinreduces 62-85% ($P<0.001$) edema due to benzoyl-peroxidase in SENKAR mouse skin.Other results of this study has shown that in the presence ofSilymarinmyeloperoxidase activity reduces 42-100% ($P<0.001$) and interlukin-1α protein level reduces 36-81% ($P<0.001$) in the epidermis.

Hammerberg *et al*. [13] studied evaluation of Silymarin anti-inflammatory effects surveyed $CD11b^+$ cells infiltration levels with anti-antibodies of this cells. Results showed that in Silymarin treated group, infiltration of these cells to the exposure area with UV radiation 59% are decreased. $CD11b^+$ cells are as an indicator of neutrophil and macrophage presence level in inflammation area. In fact Silymarin inhibits tissue over damage with inhibition from these cells infiltration. Inanothersimilarstudy that was conducted by Katiyar *et al.* [14] determined that Silymarin intra peritoneal injection reduces the destructive effects of radiation. Theresults are obtained using distributed $CD11b^+$ level measurement between skin tissue (epidermis and dermis).

Katiyar *et al*. [9] used 900mJ/cm^2 UV-B radiation during 48 hours to create acute skin inflammation due to UV-B radiation and comparison with Silymarin treated group. The incidence and severity of damages demonstrated a significant reduction in treatment group with Silymarin (71%, $P<0.001$).

Chatterjee *et al*. [15] in their study used 180mJ/cm^2 UV-B radiation for 10 days to create skin chronic inflammation. Histopathological results after this period, Indicate lesions such as hyperkeratosis, akantose, crust and lymphocyte, monocyte and eosinophil infiltrationin to the dermis. The group had received topical Silymarin, showed 92 % damage rate reduction and this reduction in group that received Silymarin orally, was 73 percent.

In our study, UV-B radiation (180mJ/cm^2) was used for 10days as an inflammation causeto create chronic inflammation in the shaved area. Histological damages in 1 and 2 control groups (not treated with Silymarin) were: hyperkeratosis, exocytose, lymphocyte infiltration to the sebaceous gland, edema and lymphocyte, plasma cell and eosinophil infiltration into the dermis. In treatment group1 that Silymarin with acetone were used locally on the skin, damage significant rate decreased significantly (79%). Also, damages were less severe than the control group's damages. Thesedamages include: edema, increased thickness of dermis and lymphocyte, plasma cell and eosinophil infiltration into the dermis. It should be mentioned that number of animals in treatment group1 that showed this damages, was accompanied with 67% decrease. Damage incidence rate decreased 58 % in treatment group 2 that were only in standard nutritional and environmental conditions. Severity of damages in treatment group 2, include hyperkeratosis, edema, increased thickness of dermis and lymphocyte, plasma cell and eosinophil infiltration into the dermis was less severe than controlgroup 2. Also, number of animals in treatment group2 that showed this damages had a 50% decrease in comparison with number of animals with similar damages in group 2.

Damages incidence comparison between treatment group1 and treatment group 2 showed 21% decrease in treatment group 1. This indicates that topical use of

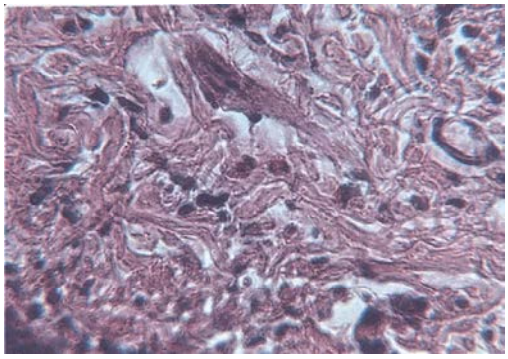

Fig. 1: Sever infiltrationof Lymphocyte, plasmacell and eosinophilto dermal area in the control groups (very high impact of Cells are stained with H&Einfiltrated and caused edema in derm ×400)

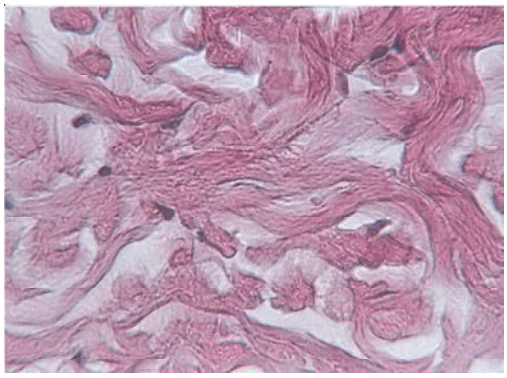

Fig. 2: Low infiltration of Lymphocyte, plasmacellor eosinophil to dermal area in the treatment groups (very lowimpact of Cells are stained with H&Ederm ×400)

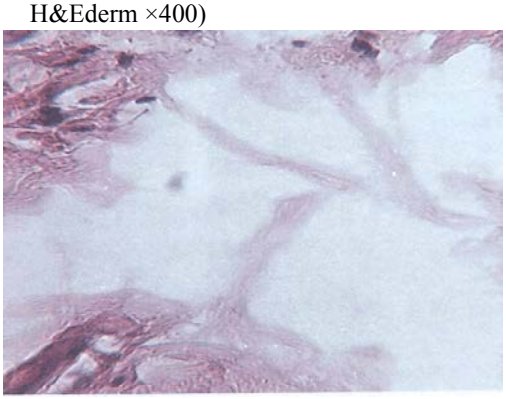

Fig. 3: Sever edema in the control groups (sever edema in extracellular of Derm stained with H&E ×400)

Silymarin in comparison with oral use has a stronger inhibitory effect in preventing from skin damages due to UV radiation. In other words, Silymarin anti-inflammatory effects against UV radiation is very effective and more evident if be used locally. However, according to previous studies, Silymarin effect is also significant when used orally. Comparison between damage incidence rates, severity of damages and number of animals with these damagesdid not show a significantly differences between control groups 1 and 2. This indicates that acetone as an article stimulus not has an effective role in severity and incidence rate of skin damages due to UV radiation (Figures 1, 2 and 3).

This study showed that Silymarin anti-inflammatory effects are considerable in skin protection against UV radiation damages, especially when used topically. In recent years, studies have shown that Silymarin has anti-inflammatory, antioxidant and anticancer effects. These features include protection against sunburn, DNA damage, Non-melanoma skin cancer and Immune suppression. These properties will bepromising use of this plant material for completing and improving protective effects of current sunscreen creams. Because there is no sun block cream that can be completely protective against different UV spectrum.

CONCLUSION

Silymarin, especially in topical administration, have significant protective effects on UV lesions. This study showed that Silymarin anti-inflammatory effects are considerable in skin protection against UV radiation damages, especially when used topically. However, more studies still need for human consumption effects of this drug in long-term, cell receiving amounts, tissue distribution. Dosage determination and how the optimum use, in order to receive the best results from beneficial effects of Silymarin.

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