ORIGINAL RESEARCH

The effect of silymarin on the liver in thermal burn injury

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ABSTRACT

Hepatic homeostasis and metabolism are essential for survival in critically ill and severely burned patients. Silymarin, the major component of thistle milk has been shown to have hepatoprotective effect. In this study the effect of silymarin on the liver of burned rats was investigated. Wistar Albino rats were exposed to 90^0C bath for 10 seconds to induce skin burn. Silymarin either locally (30 mg/kg) applied on 4 cm^2 area or locally+systemically (50 mg/kg p.o) was administered after burn and repeated twice daily. Rats were decapitated 48 h after injury and blood was collected for tumor necrosis factor-α (TNF- α) and lactate dehydrogenase (LDH) activity. In liver tissue samples, total protein, tissue factor activity (TF) and activities of carbonic anhydrase (CA), glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), alkalen and acid phosphatase (ALP, ACP respectively) enzymes were determined. In addition polyacyrlamid gel electrophoresis was carried on liver tissue samples. Burn caused a significant increase in blood TNF- α and LDH levels. Total protein levels and ALP activity increased and SOD activity decreased in the liver tissue at 48h after burn. Both local and systemic silymarin treatment significantly reversed this parameters except ALP. Silymarin treatment significantly increased ALP activity. Local silymarin treatment increased, CAT and ACP activities and decreased TF activity compared to control and burn group; and increased CA activity compared to systemic+local silymarin treatment. Addition of systemic silymarin treatment to local silymarin treatment reversed these effects of local treatment to control group levels. Non significant differences were found between protein bands obtained in electrophoresis. Minor liver damage was obvious 48 h after thermal skin burn. Both local and systemic silymarin treatment were effective to reverse the effects of burn induced liver injury.

Key Words: Burn, liver enzymes, liver tissue factor acti-vity, SDS-polyacrylamide gel electrophoresis, silymarin

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INTRODUCTION

Thermal trauma, one of the most common problems faced in the emergency room, may cause damage to multiple organs distant from the original burn wound and may lead to multiorgan failure (1). Following burn injury all tissues subject to ischemia and consequently, during burn shock resuscitation, reperfusion occurs. The role of oxygen radicals, neutrophils and endothelial cells in ischemic insult or organ failure is well documented (2, 3).

The liver plays an important role in the body's response to thermal injury. It is the principal organ responsible for producing acute-phase proteins and modulating the systemic inflammatory response (4,5). Following thermal injury, the acute-phase response brings about the activation of the coagulation and complement cascades, granulocytes, and monocytes/ macrophages as well as platelets (6-10) and induces the liver to synthesize and release proteins that exert effects on a variety of tissues (1, 4, 5).

Burn injury induces also many pathological changes in the human body, leading to alterations in pharmacokinetic parameters such as bioavailability, protein binding, volume of distribution and clearance (11). The pharmacokinetic parameters following burn injury vary between the acute phase of injury and the second hypermetabolic phase beyond the initial 48 hours of thermal injury (12). The effects of systemic and local antioxidant treatment on severe burn have been extensively studied (13). The results showed that prevention of local tissue damage is an important issue since skin burn may cause damage to multiple organs distant from the original burn wound and may lead sepsis and multiple organ failure (14).

The extracts of the flowers and leaves of *Silybum marianum* (St. Mary's thistle, milk thistle) have been used for centuries to treat liver, spleen and gallbladder disorders. In the 1960s the biologically active molecules of the seed and fruit extracts were isolated, and the chemical structures were elucidated. The isolation led first to a mixture that was named silymarin, and it was this flavonolignan mixture, with that most of the clinical studies were carried out. Flavanoids are naturally occurring substances that possess various pharmacological actions and therapeutic applications. Some of these, due to their phenolic structures, have antioxidant effect and inhibit free radical-mediated processes (15-17).

Based on these findings, we investigated the putative protective role of local and systemic (oral) silymarin treatment against burn-induced oxidative and degenerative damages on the liver at the late phase of burn.

MATERIALS AND METHODS

Animals and Protocol for the Induction of Burn

Wistar albino rats of both sexes, weighing 250 to 300 g, were fasted for 12 h, but allowed free access to water before the experiments. The animals were kept in individual wire-bottom cages, in a room at a constant temperature (22 ± 2ºC) with 12-h light and dark cycles, and fed standard rat chow. The study was approved by the Marmara University School of Medicine Animal Care and Use Committee.

The rats were divided into the following four groups of six rats (three males and three females) in each; 1- vehicle-treated sham operated control group, 2- vehicle treated burn group , 3- local silymarin-treated burn group and 4- local+oral silymarin-treated burn group

Under brief ether anesthesia, the dorsum of the rats was shaved, and exposed to 90 ºC water bath for 10 sec, which resulted in partial-thickness second-degree skin burn involving 30% of the total body surface area. All the burned animals were then resuscitated with physiological saline solution (10 ml/kg, subcutaneously, s.c.). In order to rule out the effects of anesthesia, the same protocol was applied in the control group, except that the dorsums were dipped in a 25 ºC water bath for 10 sec.

Silymarin Treatment

Silymarin either locally (30 mg/kg) applied on 4 cm^2 area or locally+oral (50 mg/kg p.o) was administered after burn and repeated twice daily.

Rats were decapitated 48 h after injury and blood was collected for tumor necrosis factor-α (TNF- α) and lactate dehydrogenase (LDH) activity. In liver tissue samples, protein, tissue factor (TF) activity and activities of carbonic anhydrase (CA), glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), alkalen and acid phosphatase (ALP, ACP) enzymes were determined. In addition polyacyrlamid gel electrophoresis was carried on liver tissue samples.

Biochemical assays
Serum TNF-α and LDH analysis

Serum TNF-α and LDH were quantified according to the manufacturer's instructions and guidelines using enzyme-linked immunosorbent assay (ELISA) kits specific for the rats (Biosource International, Nivelles, Belgium).

Livers Tissue Analysis

Liver tissue total protein, TF activity and CA, GST, CAT, SOD, ALP, ACP enzyme activities were determined by the methods of Lowry (18), Ingram (19), Vepoorte (20), Habig (21), Aebi (22), Mylorie (23), Walter (24) respectively. Electrophoretic examination of liver proteins was carried out by Laemmli SDS-polyacrylamid gel electrophoresis (25).

Statistical Analysis

Statistical analysis was done using SPSS 10.0. All data are expressed as means ± S.D. Kolmogorov-Smirnov test, Friedman test, Mann-Whitney-U test and Spearsman correlation analysis were used.

RESULTS

Burn caused a significant increase in blood TNF- α and LDH levels (Table 1). Total protein levels and ALP activity increased and SOD activity decreased in the liver tissue at 48h after burn (Table 2). Both local and systemic silymarin treatment significantly reversed this parameters except ALP. Silymarin treatment significantly increased ALP activity. Local silymarin treatment increased, CAT and ACP activities and decreased TF activity compared to control and burn group; and increased CA activity compared to systemic+local silymarin treatment. Addition of systemic silymarin treatment to local silymarin treatment reversed these effects of local treatment to control group levels. Non significant differences were found between protein bands obtained in electrophoresis (Fig1).

Table 1: Blood TNF-α and LDH levels

	Control group	Burn group	Burn Treatment Groups	
			Local silymarin	Local+oral silymarin
TNF-α (pg/mL)	4,00± 1,58	23,20 ± 5,63[a]	10,00±3,39[b]	7,60±2,07[b]
LDH (U/L)	2514±316,80	4238±375,90[a]	2854±475,90[b]	2666±429,20[b]

Values are given mean±***standard deviation;*** [a] p<0,01 significantly different from control group; [b] p<0,01 significantly different from burn group

Table 2 : Activities of glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), carbonic anhydrase (CA), tissue factor (TF), acid phosphatase (ALP), alkalen phosphatase (ACP) enzymes and protein levels in liver tissue samples of all groups (n=6 per group)

			Burn Treatment Groups	
	Control	**Burn**	**Local Silymarin**	**Local+Oral Silymarin**
GST (U/ mg protein)	0,46±0,4	0,42±0,8	0,47±0,7	0,49±0,2
CAT (U/ mg protein)	30,06±9,4	36,81±6,7	52,88±6,1[b,d ,e]	40,22±9,1
SOD (U/ mg protein)	1,21±0,7	0,84±0,2[b]	1,08±0,8[a,c]	0,96±0,2[b]
CA (U/ mg protein)	0,23±0,26	0,24±0,29	0,26±0,36[e]	0,21±0,25
TF (sec)	181,5±15,7	188,5±27,5	316,6±51,3[a,c,e]	180,8±21,0
ACP (U/ mg protein)	33,48±3,01	31,91±3,60	41,34±4,65[b,d,e]	39,08±4,23
ALP (U/ mg protein)	0,89±0,18	1,32±0,29[a]	1,97±0,42[b,d,e]	1,59±0,21
Total protein (mg/ mg tissue)	217,5±17,6	253,7±9,9[b]	210,3±7,9[d]	214,8±8,41[d]

Values are given mean±standard deviation. [a]p<0,05 and [b]p<0,01: significantly different from control group; [c]p<0,05 and [d]p<0,01: significantly different from burn group; [e]p<0,05 and [f]p<0,01: significantly different from burn+local and oral silymarin treatment groups. Since the clotting time is inversely proportional to the TF activity, the lengthening of the clotting time is a manifestation of decreased TF activity.

Figure 1: Samples of liver SDS-PAGE
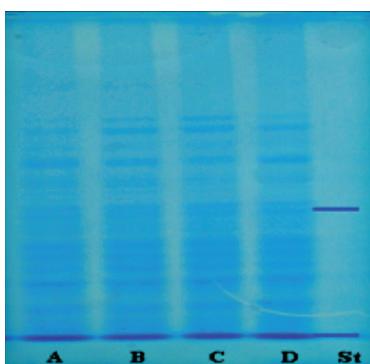
Columns: (A) burn+local+oral silymarin treatment, (B) burn+local silymarin treatment, (C) burn, (D) control, (St) Standard-Bovine Albumin

DISCUSSION

Following burn injury, increasing evidences implicated reactive oxygen species (ROS) as causative agents of local and systemic damage. ROS are first generated from the burned skin (26). Activated mononuclear phagocytes (27) and neutrophils (28,29) sequestered in systemic organs would also be the source of ROS.

It is well known that ROS give rise to damage to proteins, lipids, nucleic acids and other biological macromolecules. In addition, lipid peroxides generated from burned skin are released into the serum and diffuse into some organs causing subsequent organ injury (30). After thermal injury, uncontrolled ROS production is, at least in part, responsible for the local wound edema and systemic pathophysiological changes. The effects of systemic and local antioxidant treatment on severe burn have been extensively studied (31). The prevention of local tissue damage is an important issue since skin burn may cause damage to multiple organs distant from the original burn wound and may lead sepsis and multiple organ failure (14).

In the present study serum TNF-α and LDH markedly increased following skin burn. Silymarin treatment reduced serum levels of the proinflammatory cytokine TNF-α and LDH. These findings suggest that silymarin, appears to have a protective role in the burn-induced injury. It was demonstrated that some phytochemicals, including silymarin, are known to suppress cancer cell proliferation, inhibit growth factor signaling pathways, induce apoptosis, and inhibit NF-kappaB (32). Chang et al. who studied the mechanism of the inhibition of NF-kB, demonstrated that silymarin inhibited TNF-α-induced calcium-dependent NF-kappaB activation irrespective of its antioxidant effect (33). However, since proinflammatory cytokines are triggered by ROS, antioxidants may contribute the inhibition of cytokine release by the inhibition of radicals. Although we did not evaluate the NF-kB, it can be speculated that inhibition of proinflammatory cytokine, TNF-α may cause the inhibition of NF-kB. Furthermore, silymarin was also shown to modulate immune response, by augmenting synthesis of anti-inflammatory cytokines, such as IL-10, IL-12 (34,35).

Alteration of liver function tests is extremely common following major burns. An incidence as high as 50% has been described when there is transient elevation of the aspartate aminotransferase, alanine aminotransferase, and particularly the ALP (1). The precise etiology of these changes is not known, but they usually are benign in character and resolve spontaneously. Liver ALP activity was determined in the present study and it increased significantly in burn group consistent with other burn studies (1,36). Both silymarin treatments did not reduce this increase.

After severe burn, hepatic protein synthesis shifts from hepatic constitutive proteins, such as albumin, prealbumin, transferrin, and retinol-binding protein, to acute phase proteins (5) which serve as mediators of the inflammatory process, function as transport proteins, and participate in burn wound healing (10,37). In consistency, in the present study liver total protein level was found to be significantly increased in burn group at 48 h after burn. Both silymarin treatment reversed this effect. However no significant differences were found between protein bands obtained in electrophoresis.

SOD enzyme is widely distributed in various tissues, but it can be found especially in the liver (38). SOD catalyzes the dismutation of two superoxide radicals to O_2 and H_2O_2 (39). Liver SOD activity was found to be significantly decreased in burn group in this study. The decrease of SOD activity in the liver has been shown in thermal burn studies (40, 41, 42). In our study silymarin treatments significantly decreased SOD activity in liver.

In a recent study by Ramakrishnan et al. (43), investigating the mechanism underlying the protective effects of silymarin in hepatic carcinogenesis, it was demonstrated that a number of

endogenous antioxidants, including GSH, SOD, CAT, glutathione peroxidase (GPx), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PD) can be induced by silymarin, and that this chemically mediated upregulation of cellular defenses is accompanied by a markedly increased resistance to hepatic cell injury elicited by ROS (43). Silymarin also reduces the increase of hepatic stellate cells and TGF-1β production in the CCl$_4$-treated rats suggesting that silymarin prevents hepatic fibrosis through suppression of inflammation and hypoxia in the hepatic fibrogenesis (44). Moreover, oral silymarin administration has been shown to reduce lipid peroxidation of brain tissues in acetaminophen induced toxicity by enhancing antioxidant enzymes (45). On the other hand silymarin improving antioxidant status in blood and liver, positively affects plasma lipoprotein profile in an experimental model of dietary induced hypertriglyceridemia (46).

In addition, thermal injury has a direct impact on hepatic metabolism: the rate of phase I metabolism decreases, which is thought to be related to the oxygen-derived free radicals released during the course of injury, whereas phase II metabolism is unimpaired and may possibly increase (12). Altered phase I and phase II metabolism are not only markers for liver injury but may compound liver damage as administered drugs can not be adequately cleared.

GSTs are a family of phase II enzymes that detoxify metabolites and foreign compunds by conjugating them with GSH. Although GSTs are widely distributed in many organs, liver cytosolic GSTs are considered most important in this detoxification process. In addition to their conjugation properties, GSTs also exibit some peroxidase activity, which provide protection against reactive oxygen species and peroxidized lipid products. GSTs contribute to liver homeostasis and are released into plasma after liver injury. Pham et al. found reduction in expression of specific GST enzymes after burns and found correlation with reduced GST activity. They suggested that altered GST expression may contribute to liver damage after systemic injury (47). In the present study, GST activity did not significantly change after burn injury and silymarin administration also did not alter the results. The decreases in SOD activity and increases in ALP activity that were not accompanied by significant changes in other liver enzyme activities such as GST, CAT, CA and ACP, may indicate the presence of minor damage in liver tissue at48 h after thermal burn injury. Local silymarin treatment increased, CAT and ACP activities compared to control and burn group; and increased CA activity compared to systemic+local silymarin treatment. Addition of systemic silymarin treatment to local silymarin treatment reversed these effects of local treatment to control group levels.

The present study, for the first time, investigates the effect of silymarin on liver TF activity. No significant change was found in TF activity in liver samples following burn injury. This may also support minor liver damage at 48 h after burn. TF, known as thromboplastin or Factor III, is an important coagulation factor that initiates extrinsic blood coagulation with FVII. It is not actively found in the blood but it is the cell component of the membranes (48). It has been shown that some tissues and fluids of the body have TF activities (49-54). Thrombotic and fibrinolytic mechanisms are activated after burn injury, with the extent of activation correlated to the severity of the thermal injury (5). The liver produces most of the factors involved in coagulation and fibrinolysis that help to maintain the balance between thrombin deposition and removal (1). On the other hand, TF activity decreased significantly in rats treated with local silymarin administration in the present study. Locally administered silymarin decreased the activity more effectively than locally + orally administered silymarin.This finding was also another evidence that silymarin has protective effects on liver. Addition of systemic silymarin treatment to local silymarin treatment reversed these effects of local treatment to control group levels.The effect of local + oral administration of silymarin on liver TF activity need further investigation.

CONCLUSION

Minor liver damage was obvious 48 h after thermal skin burn. Silymarin treatment was effective to reverse the damages seen after burn injury.

Termal yanıkda silmarinin karaciğer üzerine etkisi

ÖZET

Karaciğer yanık hastaları için hayati önem taşır. Meryamana dikeninin ana maddesi olan silmarinin karaciğer üzerindeki koruyucu etkisi gösterilmiştir. Bu çalışmada termal deri yanıklı sıçanlarda silmarinin karaciğer üzerine etkisi araştırıldı. Wistar-Albino türü sıçanlar 90ºC deki su ile 10 saniye temas ettirilerek deri yanığı oluşturuldu. Silmarin hem lokal (30 mg/kg) olarak hemde lokal + oral yolla (50 mg/kg) yanık oluşturulduktan sonra 2 gün (48 saat) süre ile günde 2 kez uygulandı. 48 saat sonunda sıçanlar dekapite edildi ve kan örneklerinde tümör nekroz faktör (TNF-α) ve laktat dehidrogenaz (LDH) aktivitesi incelendi, karaciğer dokusunda protein, doku faktörü (DF) aktivitesi, karbonik anhidraz (CA), glutatyon-S-transferaz (GST), katalaz (CAT), süperoksid dismutaz (SOD), alkalen fosfataz (ALP), asit fosfataz (ACP) gibi enzim aktiviteleri tayin edildi. Ayrıca SDS-poliakrilamid jel elektroforezi uygulandı. Termal yanıktan 48 saat sonra yanık grubunda TNF-α ve LDH seviyelerinde artış, karaciğer dokusunda protein ve ALP aktivitesinde artma, SOD aktivitesinde azalma tespit edildi. Lokal ve oral silmarin tedavisi bu parametrelerdeki değişimleri ALP hariç geri döndürdü, silmarin tedavisi ALP aktivitesini arttırdı. Lokal silmarin tedavisi CAT aktivitesini ve DF aktivitesini kontrol grubuna göre arttırdı. CA aktivitesini de lokal+oral silmarin tedavisi uygulanan gruba göre arttırdı. Lokal silmarin tedavisinin sistemik silmarin tedavisi ile desteklenmesi lokal silmarin tedavisinin neden olduğu bu etkileri düzeltti ve kontrol grubu düzeylerine indirdi. Elektroforez ile elde edilen protein bantları arasında anlamlı farklılıklar tespit edilmedi. Termal yanıklardan 48 saat sonra karaciğerde minor hasar meydana geldi. Silmarin tedavisi bu hasarı geri döndürmede etkili oldu.

Anahtar kelimeler Karaciğer doku faktörü aktivitesi, Karaciğer enzimleri, SDS-poliakrilamid jel elektroforezi, Silmarin, Yanık

REFERENCES

1. Price LA, Thombs B, Chen CL, Milner SM. Liver disease in Burn injury: Evidence from a National sample of 31338 adults patients. J Burns Wounds 2007; 7:1-11.

2. Schwacha MG, Somers SD. Thermal injury induced enhancement of oxidative metabolism by mononuclear phagocytes. J Burn Care Rehabil 1999;20:37-42.

3. Supple KG. Physiological response to burn injury. Crit Care Nurs Clin North Am 2004; 16:119-26.

4. Jeschke MG, Micak Rp, Finnerty CC, Herdon DN. Changes in liver function and size after a severe thermal injury. Shock 2007;28:172-7.

5. Jeschke MG. The hepatic response to a thermal injury. In: Total Burn Care. Ed. Hemdon DN, WB Saunders. London. 2002, pp.361-378.

6. Bartlett RH, FongSW, Marrujo G, Hardeman T, AndersonW. Coagulation and platelet changes after thermal injury in man. Burns 1981;7:370-7.

7. Eurenius K, Mortensen PF, Meserol PM, Curreri PW. Platelet and megakaryocyte kinetics following thermal injury. J Lab Clin Med 1971;79:147–57.

8. Eurenius K, Rossi TD, McEuen DD, Arnold J, McManus WF. Blood coagulation in burn injury. Proc Soc Exp Biol Med 1974;147:878-82.

9. Xia ZF, Coolbaugh MI, He F, Herdon DN, Papaconstantinou J. The effects of burn injury on the acute phase response. J Trauma 1992; 32:245-51.

10. Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. Immunol Today 1997; 18:428-32.

11. Jaehde U, Sorgel F. Clinical pharmacokinetics in patients with burns. Clin Pharmacokinet 1995; 29:15–28.

12. Bonate PL. Pathophysiology and pharmacokinetics following burn injury. Clin Pharmacokinet 1990; 18:118-30.

13. Tunali T, Sener G, Yarat A, Emekli N. Melatonin reduces oxidative damage to skin and normalizes blood coagulation in a rat model of thermal injury. Life Sci 2005;76: 1259-66.

14. Çakır B Cevik H, Contuk G, Ercan F, Eksioglu-Demiralp E, Yegen BC. Leptin ameliorates burn induced multiple organ damage and modulates postborn immune response in rats. Regul Pep 2005;125:135-44.

15. Editor: Montvale NJ, Medical Economics Company. 2000, pp.516-20.

16. Wellington K, Jarvis B. Silymarin: A review of its clinical proporties in the management of hepatic disorders. Biodrugs 2001;15: 465-89.

17. Kidd P, Head K. A review of the bioavailability and clinical efficacy of milk thistle phytosome: A silybin-phosphatidylcholine complex (Siliphos). Altern Med Rev 2005; 10:193-203.

18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951;193:265-75.

19. Ingram GIC, Hills M. Reference method for the one stage prothrombin time test on human blood. Thromb Haemostas 1976;36: 237-38.

20. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. J Biol Chem 1967; 242: 4221-9.

21. Habig WH, Jacoby WB. Assays for differentation of glutathion-s-transferases. Methods Enzymol 1981;77:398-405.

22. Aebi H.Catalase Invitro. In: Methods of Enzymatic Analysis. Ed: Bergmeyer HU, John Wiley & Sons. USA. 1974, pp. 121-26.

23. Mylorie AA, Collins H, Umbles C, Kyle J. Erythrocyte superoxide dismutase activity and other parameters of cupper status in rats ingesting lead acetate. Toxicol Appl Pharmacol 1986;82: 512-520.

24. Walter K, Schült C.Acid and alkaline phosphatase in serum (two point method). In: Methods of Enzymatic Analysis. Ed: Bergmeyer HU, John Wiley & Sons. USA. 1974, pp.856-86.

25. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-5.

26. Ding HQ, Zhou BJ, Liu L, Cheng S. Oxidative stress and metallothionein expression in the liver of rats with severe thermal injury. Burns 2002; 28: 215-21.

27. Schwacha MG, Somers SD. Thermal injury induced enhancement of oxidative metabolism by mononuclear phagocytes. J Burn Care Rehabil 1999;20:37–41.

28. Cetinkale O, Kenukoglu D, Senel O, Kemerli GD, Yazar S. Modulating the function of neutrophils and lipid peroxidation by FK-506 in a rats model of thermal injury. Burns 1999;25:105-12.

29. Sayeed MM. Neutrophil signaling alteration: an adverse inflammatory response after burn shock. Medicina (B Aires) 1998;58:386-92.

30. Nishigaki I, Hagihara M, Hiramatsu M, Izawa Y, Yagi K. Effect of thermal injury on lipid peroxide levels of rat. Biochem Med 1980;24:185–9.

31. Toklu HZ, Tunali-Akbay T, Erkanlı G, Yüksel M, Ercan F, Şener G.Silymarin, the antioxidant component of *Silybum marianum*, protects against burn-induced oxidative skin injury. Burns 2007;33:908-16.

32. Dorai T, Aggarwal BB. Role of chemopreventive agents in cancer therapy. Cancer Lett 2004;215:129-40.

33. Chang JW, Kim CS, Kim SB, Park SK, Park JS, Lee SK. Proinflammatory cytokine-induced NF-kappaB activation in human mesangial cells is mediated through intracellular calcium but not ROS: effects of silymarin. Nephron Exp Nephrol 2006;103:156-65.

34. Schumann J, Prockl J, Kiemer AK, Vollmar AM, Bang R, Tiegs G. Silibinin protects mice from T cell-dependent liver injury. J Hepatol 2003;39:333–40.

35. Meeran SM, Katiyar S, Elmets CA, Katiyar SK. Silymarin inhibits UV radiation-induced immunosuppression through augmentation of interleukin-12 in mice. Mol Cancer Ther 2006;5:1660-8.

36. Latha B, Ramarrishnan M, Jayaraman V, Babu M.Serum enzymatic changes modulated using trypsin:chymotrypsin preparation during burn wounds in humans. Burns 1997;23: 560-4.

37. Jeschke MG, Herndon DN,Wolf SE, et al. Recombinant human growth hormone alters acute phase reactant proteins, cytokine expression, and liver morphology in burned rats. J Surg Res 1999; 83:122-9.

38. Greenwald RA.Superoxide dismutase and catalase as therapeutic agents for human disease. A critical review. Free Radic Biol Med 1990; 8:201-9.

39. Guemouri L, Artur Y, Herbeth B, Jeandel C, Cuny G, Siest G. Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. Clin Chem 1991;37:1932-1937.

40. Kawai S, Komura J. Experimental burn-induced changes in lipid peroxidelevels, and activity of superoxide dismutase and glutathione peroxidase in skin lesions,serums, and liver of mice. Arch Dermatol Res 1988;280:171-5.

41. Naziroglu M, Kokcam I, Yilmaz S.Beneficial effects of intraperitoneally administered alpha-tocopheryl acetate on levels of lipid peroxide and activity of glutathione peroxidase and dismutase in skin, blood and liver of thermally injured guinea pigs. Skin Pharmacol Appl Skin Physiol 2003;16:36-45.

42. Saitoh D, Okada Y, Ookawara T, Yamashita H, Takahara T, Ishihara S, Ohno H, Mimura K. Prevention of ongoing lipid peroxidation by wound excision and superoxide dismutase treatment in the burned rat. Am J Emerg Med 1994;12: 142-6

43. Ramakrishnan G, Raghavendran HR, Vinodhkumar R, Devaki T. Suppression of N-nitrosodiethylamine induced hepatocarcinogenesis by silymarin in rats. Chem Biol Interact 2006;161:104–14.

44. Jeong DH, Lee GP, Jeong WI, Do SH, Yang HJ, Yuan DW, et al. Alterations of mast cells and TGF-beta1 on the silymarin treatment for CCl(4)-induced hepatic fibrosis. World J Gastroenterol 2005;11:1141-8.

45. Nencini C, Giorgi G, Micheli L. Protective effect of silymarin on oxidative stress in rat brain. Phytomedicine 2007;14:129-35.

46. Skottova N, Kazdova L, Oliyarnyk O, Vecera R, Sobolova L, Ulrichova J. Phenolics-rich extracts from Silybum marianum and Prunella vulgaris reduce a high-sucrose diet induced oxidative stress in hereditary hypertriglyceridemic rats. Pharmacol Res 2004;50:123-30.

47. Pham TN, Cho K, Warren AJ, Greenhalgh GD. Reduced hepatic expression of glutathion S-transferases in burned mice. J Trauma 2004;57:867-71.

48. Bachlie E. History of thromboplastin. Br J Haematol 2000;110:248–55.

49. Ashkinazi IIa. Functional deficiency of erythrocyte tissue factor. Bull Exp Biol Med 1997;83:664–6.

50. Lwaleed BA. Tissue factor:biological function and clinical significance. Saudi Med J 2002;23:135–43.

51. Tutuarima JA, Hische EAH, Trotsenburg L, Helm HJ. Thromboplastic activity of cerebrospinal fluid in neurological disease. Clin Chem 1985;31: 99-100.

52. Yarat A, Tunali T, Pisiriciler R, Akyuz S, Ipbuker A, Emekli N. Salivary thromboplastic activity in diabetics and healthy controls. Clin Oral Investing 2004;8: 36-9.

53. Emekli-Alturfan E, Kasikci E, Yarat A. Tissue factor activities of streptozotocin induced diabetic rat tissues and the effect of peanut consumption. Diabetes Metab Res Rev 2007;23: 653-8.

54. Aktop S, Emekli-Alturfan E, Ozer C, Gonul O, Garip H, Yarat A, Goker K. Effects of Ankaferd Blood Stopper and Celox on the tissue factor activities of warfarin-treated rats. Clin Appl Thromb Hemost 2014;20: 16-21.