



Vitamin E Effects on Intestinal Damages in Burned Rats

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ABSTRACT

Background: Vitamin E is a fat-soluble agent protecting cells from free radicals damages. Previous studies have shown that oxidative stress plays an important role in mucosal intestinal damages in burn trauma. This study aimed to investigate vitamin E effects on small intestinal mucosal changes in burned rats. **Methods:** Mature male rats (n=32) weighing 260 ± 10 g were used in this experiment. After induction of deep general anesthesia, a determined area of rats' back region (10% of body surface) was exposed to 95°C water for 8 seconds to induce a second-degree wet burn. The evaluated groups in our study were: 8 rats without burning, 8 rats without burning treated with vitamin E, 300 mg/kg/day for 15 days, 8 burned rats without medication and 8 burned rats treated with vitamin E, 300 mg/kg/day for 15 days. All rats were killed on fifteenth day by ether inhalation. The samples were taken from the first part of small intestine and were stained by Hematoxylin & Eosin method. **Results:** Burned rats receiving vitamin E had a higher intestinal villi height and lower intestinal lumen diameter as compared to burned rats without the vitamin E treatment ($P < 0.05$ and $P < 0.01$, respectively) and those values were close to the results of unburned ones. There were no significant differences among the study groups regarding the intestinal diameter and muscular layer thickness. **Conclusion:** Vitamin E can improve intestinal villus height and lumen diameter and its consumption at the time of burning may protect intestine mucosa.

Introduction

Burn injury is associated with a high incidence of death and disability.¹ Fires cause 1% of the global burden of diseases² and 300,000 deaths per year.³ The leading causes of death in burn patients are multiple organ failure and infection.⁴ In serious burns, pathologic and physiologic changes are not limited to skin and affect the whole body systematically.^{5,6} There is a significant decrease in organ blood flow, which can be complicated by sepsis and multi-organ failure.^{7,8} Intestine, kidney and liver are the organs most affected by prolonged tissue hypoxia and reperfusion injury.^{9,10} Shortly after thermal injuries, approximately 6 hours, a significant vasoconstriction of omental arterioles induces apoptosis of small bowel epithelium and causes increased cell death.¹¹⁻¹³ In addition, shortened intestinal villi and reduced DNA contents have been observed in burned patients.¹⁴ Oxygen free radicals such as superoxide anions and hydroxyl radicals are produced by lipid peroxidation after burn.¹⁵ It is believed that these radicals play an important role in

development of burn shock and multiple organ injury.¹⁶⁻¹⁸ Stopping oxidative products generation has been shown to enhance the rate of epithelialization.¹⁹ The antioxidants can scavenge the free radicals that are produced during the burn stress.²⁰ This is especially true of Vitamin E.^{21,22} It was reported that the burn patients have depleted stores of Vitamin E.²³ These antioxidants may reduce the lipid peroxidation²¹ in intestinal cells by reducing the free radical contents. The aim of this study was the evaluation of vitamin E effects on small intestine mucosal changes in burned rats.

Methods and Materials

Animal models

Thirty-two Sprague-Dawley male rats (four months old, body weight 250-270 g) were used in this experiment. The animals were housed in metal cages in a temperature-controlled room (25 ± 2 °C) with a 12-hour light/dark cycle (lights on at 8:00 A.M.). Standard rat

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chow and water were freely available. All experiments were performed in accordance with institutional guidelines for animal care and use and also "Principles of laboratory animal care" were followed.

Study groups

The animals were randomly divided into four groups (C, CE, B and BE), each consisting eight rats (n=8). The evaluated groups of this study were: rats without burning and medication (Control: unburned rats that shaved their back and received water by gavage as vehicle) rats without burning treated with vitamin E (CE: Unburned rats that shaved their back and received Vitamin E by gavage), burned rats without medication (B: Burned rats that received water by gavage) and burned rats treated with vitamin E (BE: Burned rats that received Vitamin E by gavage). We used water - miscible vitamin E from Merck Co. with product number of 5008621000, so we used water as solvent. Rats in burn groups experienced burning trauma and were subsequently fed in the next 15 days. Animals in non-vitamin E groups were fed with standard food whilst Vitamin E groups' rats received 300 mg/kg vitamin E by gavage daily.

Anesthesia and Burning

Sixteen out of thirty-two rats of this study were burned. General anesthesia was induced by the injection of ketamine (50mg/kg) and diazepam (5mg/kg) before burning. The route of injection was intramuscular. After anesthesia, animal's back hairs were shaved and an area of 10 percent of the animal's body surface was exposed to heat. Walker's formula was used to calculate the extent of burning region according to animal's weight. An especial mold was built to precisely control the burning area. The interior surface of the mold was insulated against the heat and a 3.5×5 cm opening was made in its upper concave wall. To induce the burning stress, the mold was filled with 95°C water and animal's back region was cautiously put in the water for 8 seconds in the supine position. To prevent systemic shock, 5 ml of normal saline (18.5-20 ml/kg) was injected intraperitoneally both in control and test groups.

Sampling

The rats were initially weighted and then killed by diethyl ether inhalation on fifteenth day after the burn. After opening the peritoneal cavity, a 0.5 cm sample of the first duodenum was taken and fixed, using 10% formaldehyde in phosphate buffer (pH 7.4).

Tissue process and staining

Tissue process consists of dehydration, clearing and infiltration steps. Samples were transferred through progressively more concentrated baths of ethanol to remove water. 50%, 60%, 70%, 80%, 90% v/v and absolute alcohol were used for 30 min RT each. Xylene was then used to remove the alcohol. Finally, paraffin was used as the infiltration agent. The samples were

embedded in paraffin molds. The paraffin blocks were cut into 5-micrometer-thick sections using leitz microtome. After preparing the samples, hematoxylin and eosin (H&E) method was used for staining.

Morphometric measurements

All morphometric measurements were made in tissue sections under 10x magnification of light microscope (Leica DM5000). The microscope was equipped with a reticule, especial scale, to allow measurements. Transverse circular sections were used for morphometric measurements. The measured parameters were intestinal diameter, intestinal lumen diameter, intestinal muscular layer thickness and villi height. Layers' border determination was done according to histological definitions. The greatest and the least amounts of each parameter were measured in each section and the mean value was considered as the amount of that parameter in that section. Measurements were made in ten sections for each case and the mean value of these numbers was considered as the final amount.

Statistical analysis

Statistical package for the social sciences (SPSS for Windows, version 17 Chicago, IL, USA) was used for data analysis. All data was represented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for multiple comparisons of the groups, followed by Tuckey's post-hoc test. *P value* < 0.05 was considered as statistical significance limitation.

Results

Intestinal diameter

There were no significant differences among experimental groups in intestinal diameter. Even though burn group rats had a slightly lower intestinal diameter compared to control groups, the difference was not significant (table 1).

Mean values of intestinal diameter in experimental groups are shown in figure 1-A.

Intestinal lumen diameter

Intestinal lumen diameter was significantly higher in burn group compared to control (*p value* < 0.05) and control with vitamin E (*p value* < 0.01) groups. There was not any significant difference between burn rats treated with vitamin E and control groups (table 1). Mean values of intestinal lumen diameter in experimental groups are shown in figure 1-B.

Muscular layer thickness

No significant differences were observed among study groups in muscular layer thickness. Burn group rats had a slightly higher muscular layer thickness compared to control groups, but the difference was not significant (table 1). Mean values of muscular layer thickness in experimental groups are shown in figure 1-C.

Table 1. Comparing of vitamin E effects on small intestinal mucosal changes in burned and control rats.

Groups Variables	Control (n=8)	Control with Vit E (n=8)	Burn (n=8)	Burn with Vit E (n=8)	p value
Intestinal diameter	3200 ± 167.33	3190 ± 176.36	3150 ± 187.76	3200 ± 141.42	NS
Intestinal lumen diameter	641.67 ± 80.1	621.56 ± 82.32	848.33 ± 64.93*	583.33 ± 40.82	p < 0.01
Muscular layer thickness	94.17 ± 14.28	95.13 ± 12.46	99.17 ± 10.68	93.33 ± 10.80	NS
Villi height	950 ± 34.05	951 ± 32.21	848.33 ± 58.79*	921.67 ± 27.86	p < 0.05

Villi height

Villi height was significantly lower in burn group compared to control (p value < 0.01) and control with vitamin E (p value < 0.05) groups. There was no significant difference between burn group treated with vitamin E and control groups in villi height (table 1). Mean values of villi height in different experimental groups are shown in figure 1-D.

Discussion

This study implies that vitamin E possesses inhibitory effects on morphometric changes of the small intestinal mucosa after burn injury. In other words, it reduces cell death in small bowel epithelium.

Apparently, burn injuries damage the skin more than anywhere else in the body. But in serious burns, the body is affected systematically.⁶ Redistribution of blood from gastrointestinal viscera and kidneys to vital organs and the affected skin is the compensatory mechanisms. This redistribution, especially in prolonged periods, may have inevitable consequences for hypoperfused organs.²⁴ Gastrointestinal system is one of the most affected systems in burn patients.²⁵ Ischemia-reperfusion injury of the intestine is an important factor associated with high rates of morbidity and mortality.²⁶ One of the major functions of the gut is to prevent the absorption of toxins, antigens, proteases and microorganisms across the intestinal wall.²⁷

Severe vasoconstriction occurs in gastrointestinal vasculature after the burn injury and gastrointestinal barrier dysfunction can permit the harmful agents to enter the blood circulation.^{10,28} Septicemia is the leading cause of death in burn patients.²⁹ There are reports of as many as 30% of the burn patients who no detectable microorganisms were found in their repeated wound cultures, while their blood cultures were positive for gut flora.^{30,31} It is thought that intestinal microbial flora is the major source of this group of septicemia. So it is of great importance to save this physiologic barrier after the burn injury. Based on histological examination, it was found that the small

intestine is more susceptible to ischemia/reperfusion injury than the colon and most of the previous studies have focused on small intestine.²⁷

Several studies have suggested that hypoperfusion and ischemia/reperfusion injury of the gut, as well as the release of proinflammatory cytokines and free radicals are associated with apoptosis of the small bowel epithelium.^{28,32,33} Free radicals are very unstable molecules, which react quickly with other compounds, trying to capture the needed electron to gain stability.³⁴ To avoid this harmful electron transfer, intra and extra cellular antioxidants try to eliminate these unstable molecules. There is a regulated balance between oxidant and antioxidant agents in the body.³⁵ Oxidative stress induced by oxidant species, results in decline of antioxidant defense mechanisms in various organs of the burn patients.^{36,37} If the body cannot keep the balance and free radicals overcome the defense system, they will start to attack DNA, proteins and lipids that results in cellular damage.^{38,39} Free radical burst is associated with ischemia-related skin tissue injury.⁴⁰ Enhanced free radical production is accompanied by impaired antioxidant mechanisms.⁴¹ Vitamin E functions as a chain-breaking antioxidant *in vivo* and prevents the propagation of free radicals damage in biological membranes.^{42,43} It is a potent peroxy radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) in cell membranes and plasma lipoproteins.^{44,45} Vitamin E modulates smooth muscle cell proliferation, platelet adhesion and aggregation, and monocyte endothelial adhesion.⁴⁶⁻⁴⁸ In addition, vitamin E has been shown to reduce intestinal cellular damages, caused by oxidative stress, in different kinds of diseases, including diabetes and infectious diarrhea.^{49,50} Previous studies have shown that Vitamin E is depleted during cardiac diseases, as well as after burns, because of increased oxidative stress.^{23,51}

Our results are consistent with previous studies, which reported decreased intestinal villous height in post-burn patients.^{52,53} In this study, it was shown that vitamin E

Fig 1. Effects of Vitamin E on intestinal parameters in control and burn experimental groups. (A) Intestinal diameter. (B) Intestinal lumen diameter. (C) Muscular layer thickness and (D) villi height.

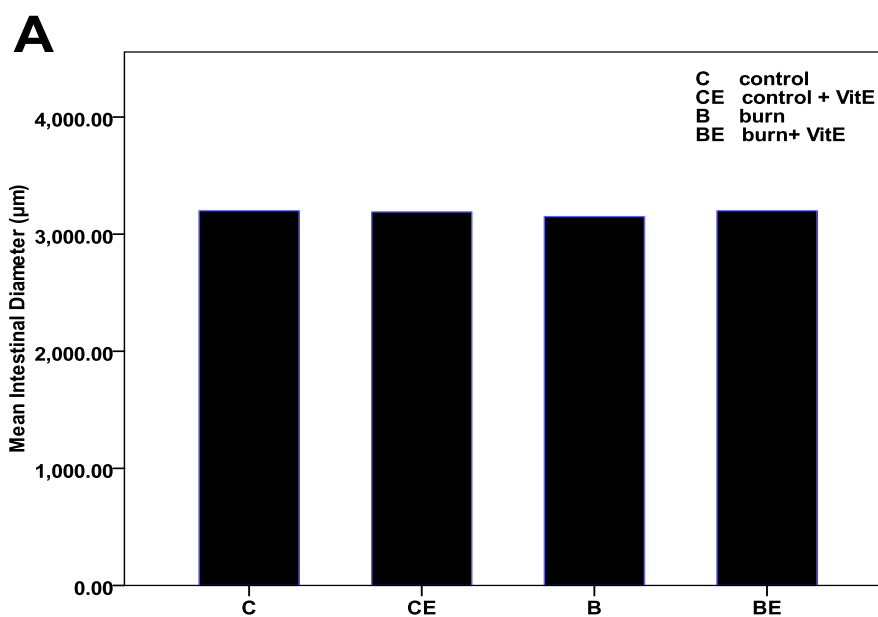


Fig 1A. Effects of vitamin E on intestinal diameter in control and burn experimental groups. * shows the significantly different group.

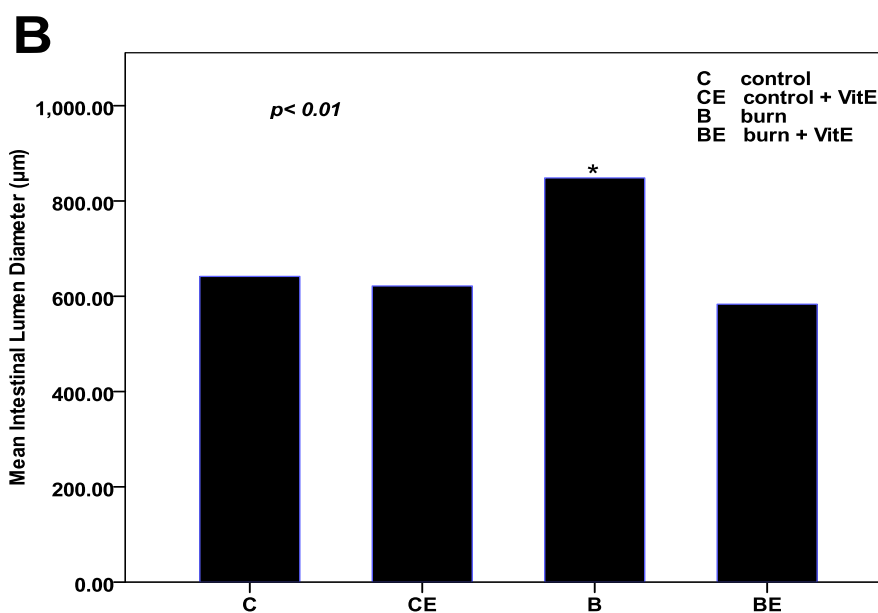


Fig 1B. Effects of vitamin E on intestinal lumen diameter in control and burn experimental groups. * shows the significantly different group.

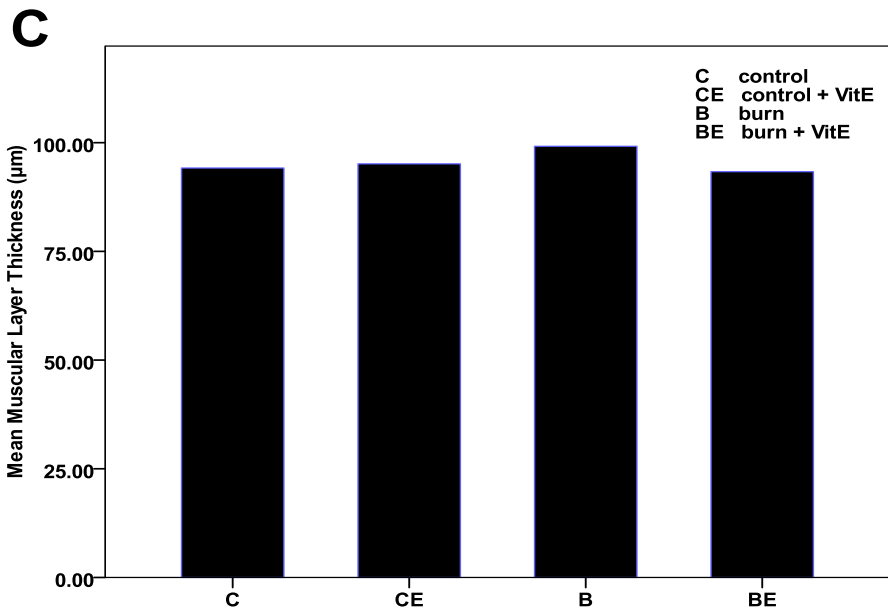


Fig 1C. Effects of vitamin E on muscular layer thickness in control and burn experimental groups. * shows the significantly different group.

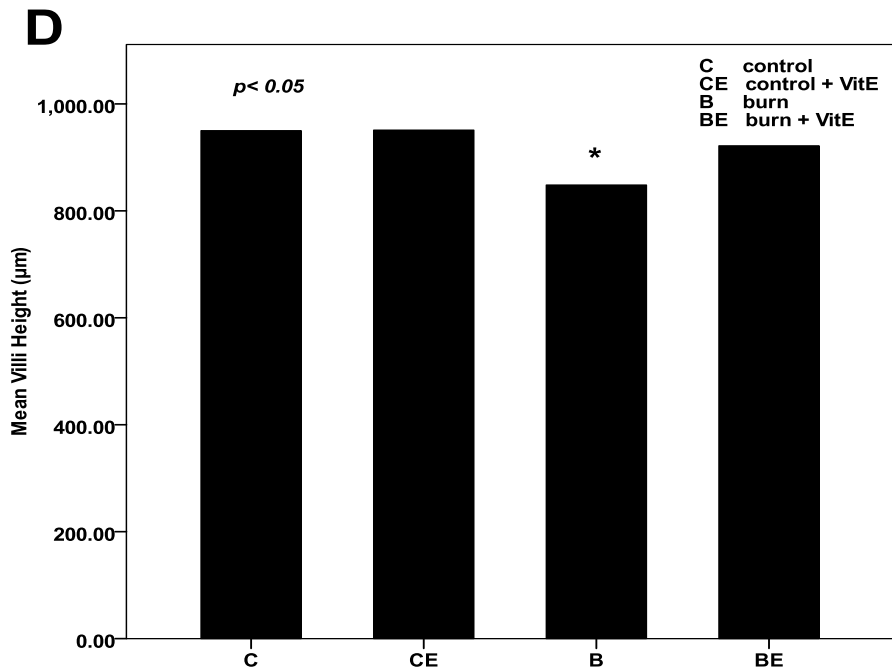


Fig 1D. Effects of vitamin E on villi height in control and burn experimental groups. villi height. * shows the significantly different group.

could reduce small intestinal mucosal injuries in burn patients. It has also helped the lumen diameter remain almost normal. Several different factors were used in previous studies to enhance the proliferation capacity of small intestinal epithelium in burn models. Growth hormone, insulin like growth factor-I and hepatocyte growth factor are among the hormonal factors that were

successfully used to reduce the intestinal mucosal damage in burned rats.^{13,54,55} To our knowledge, this is the first study that shows the beneficial effects of vitamin E on intestinal mucosa in burned rats. The mechanism by which vitamin E inhibits the epithelial cells apoptosis and enhances their proliferation is a matter of speculation. It is believed that vitamin E

influences the vascular diameter by increasing the production of vasoactive molecules, such as prostaglandin I₂ and E₂.⁵⁶ Effects of vitamin E on endothelial cells have been shown in several studies.⁵⁷ vitamin E has a regulatory effect on phospholipase A₂ and increases the production of arachidonic acid in endothelial cells.^{58,59} In addition, vitamin E is a well-known potent antioxidant agent, protecting the cells from oxidative stress.^{60,61} There is increasing evidences that oxidative stress has an important role in the development of multiple organ failure in severe burns.^{39,62} Whether the observed effect of vitamin E in this study is caused by promoting of small intestinal blood supply or by reducing destructive effects of oxidative stress is not an easy question to answer. The mechanisms of distant organs injury in severe burns are under active and continuous researches. As these mechanisms are better understood, protective effects of vitamin E are also better clarified.

In summary, the results of this research show that vitamin E can improve intestinal villuses height and lumen diameter and its consumption at the time of burning can protect intestine mucosa.

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References

1. Khorasani G, Hosseinimehr SJ, Azadbakht M, Zamani A, Mahdavi MR. Aloe versus silver sulfadiazine creams for second-degree burns: a randomized controlled study. *Surgery today* 2009;39(7):587-91.
2. Leistikow BN, Martin DC, Milano CE. Fire injuries, disasters, and costs from cigarettes and cigarette lights: a global overview. *Preventive medicine* 2000;31(2 Pt 1):91-9.
3. Peck MD. Epidemiology of burns throughout the world. Part I: Distribution and risk factors. *Burns* 2011;37(7):1087-100.
4. Kallinen O, Maisniemi K, Bohling T, Tukiainen E, Koljonen V. Multiple organ failure as a cause of death in patients with severe burns. *J Burn Care Res* 2012;33(2):206-11.
5. Rnjak J, Wise SG, Mithieux SM, Weiss AS. Severe burn injuries and the role of elastin in the design of dermal substitutes. *Tissue engineering* 2011;17(2):81-91.
6. Lund T, Bert JL, Onarheim H, Bowen BD, Reed RK. Microvascular exchange during burn injury. I: A review. *Circulatory shock* 1989;28(3):179-97.
7. Williams FN, Herndon DN, Hawkins HK, Lee JO, Cox RA, Kulp GA, et al. The leading causes of death after burn injury in a single pediatric burn center. *Critical care* 2009;13(6):R183.
8. Fang W, Yao Y, Shi Z. The time course and tissue distribution of endotoxin in rats after thermal injury. *Zhonghua zheng xing shao shang wai ke za zhi* 1999;15(4):298-300.
9. Sato Y, Itagaki S, Oikawa S, Ogura J, Kobayashi M, Hirano T, et al. Protective effect of soy isoflavone genistein on ischemia-reperfusion in the rat small intestine. *Biological & pharmaceutical bulletin* 2012;34(9):1448-54.
10. Jones WG, Minei JP, Barber AE, Fahey TJ, Shires GT, Shires GT. Splanchnic vasoconstriction and bacterial translocation after thermal injury. *The American journal of physiology* 1991;261(4):190-6.
11. Sakurai H, Traber LD, Traber DL. Altered systemic organ blood flow after combined injury with burn and smoke inhalation. *Shock* 1998;9(5):369-74.
12. Lightfoot E, Horton JW, Maass DL, White DJ, McFarland RD, Lipsky PE. Major burn trauma in rats promotes cardiac and gastrointestinal apoptosis. *Shock* 1999;11(1):29-34.
13. Jeschke MG, Bolder U, Finnerty CC, Przkora R, Muller U, Maihofer R, et al. The effect of hepatocyte growth factor on gut mucosal apoptosis and proliferation, and cellular mediators after severe trauma. *Surgery* 2005;138(3):482-9.
14. Wolf SE, Ikeda H, Matin S, Debroy MA, Rajaraman S, Herndon DN, et al. Cutaneous burn increases apoptosis in the gut epithelium of mice. *Journal of the American College of Surgeons* 1999;188(1):10-6.
15. Li X, Schwacha MG, Chaudry IH, Choudhry MA. Heme oxygenase-1 protects against neutrophil-mediated intestinal damage by down-regulation of neutrophil p47phox and p67phox activity and O₂- production in a two-hit model of alcohol intoxication and burn injury. *J Immunol* 2008;180(10):6933-40.
16. Mallikarjuna Rao C, Ghosh A, Raghothama C, Bairy KL. Does metronidazole reduce lipid peroxidation in burn injuries to promote healing? *Burns* 2002;28(5):427-9.
17. Polutova NV, Ostrovskii NV, Romantsov MG, Chesnokova NP. Positive effect of cytoflavin on metabolic status changes in patients with burn disorder. *Eksperimental'naia i klinicheskaia farmakologiya* 2011;74(7):33-7.
18. Kobayashi K, Ikeda H, Higuchi R, Nozaki M, Yamamoto Y, Urabe M, et al. Epidemiological and outcome characteristics of major burns in Tokyo. *Burns* 2005;31 Suppl 1:S3-S11.
19. Al-Jawad FH, Sahib AS, Al-Kaisy AA. Role of antioxidants in the treatment of burn

- lesions. *Annals of burns and fire disasters* 2008;21(4):186-91.
20. Fang Y, Fu XJ, Gu C, Xu P, Wang Y, Yu WR, et al. Hydrogen-rich saline protects against acute lung injury induced by extensive burn in rat model. *J Burn Care Res* 2011;32(3):e82-91.
21. Xue C, Chou CS, Kao CY, Sen CK, Friedman A. Propagation of cutaneous thermal injury: a mathematical model. *Wound Repair Regen* 2011;20(1):114-22.
22. Kim HB, Shanu A, Wood S, Parry SN, Collet M, McMahon A, et al. Phenolic antioxidants tert-butyl-bisphenol and vitamin E decrease oxidative stress and enhance vascular function in an animal model of rhabdomyolysis yet do not improve acute renal dysfunction. *Free radical research* 2011;45(9):1000-12.
23. Traber MG, Leonard SW, Traber DL, Traber LD, Gallagher J, Bobe G, et al. alpha-Tocopherol adipose tissue stores are depleted after burn injury in pediatric patients. *The American journal of clinical nutrition* 2010;92(6):1378-84.
24. Romand J, Attewell J, Pinsky M. Increases in peripheral oxygen demand affect blood flow distribution in hemorrhaged dogs. *Am J Respir Crit Care Med* 1996;153(1):203-10.
25. Siemers F, Kaun M, Machens HG, Lohmeyer JA, Mailander P. [Mesenteric ischemia: a severe complication in burn patient]. *Handchir Mikrochir Plast Chir* 2007;39(5):364-8.
26. Sato Y, Itagaki S, Oikawa S, Ogura J, Kobayashi M, Hirano T, et al. Protective effect of soy isoflavone genistein on ischemia-reperfusion in the rat small intestine. *Biological & pharmaceutical bulletin* 2011;34(9):1448-54.
27. Chang J, Chen S, Jiang L, Chen J, Chang R. Functional and morphological changes of the gut barrier during the reititution process after hemorrhagic shock. *WJ Gastroent* 2005;11:3585 - 91.
28. Jeschke MG, Bolder U, Chung DH, Przkora R, Mueller U, Thompson JC, et al. Gut mucosal homeostasis and cellular mediators after severe thermal trauma and the effect of insulin-like growth factor-I in combination with insulin-like growth factor binding protein-3. *Endocrinology* 2007;148(1):354-62.
29. Song J, Wolf SE, Herndon DN, Wu XW, Jeschke MG. Second hit post burn increased proximal gut mucosa epithelial cells damage. *Shock* 2008;30(2):184-8.
30. Xiao GX. The gut-origin infection in severe burns. *Zhonghua shao shang za zhi Chinese journal of burns* 2008;24(5):331-3.
31. Song GD. Septicemia in early stage of severe burns. *Zhonghua zheng xing shao shang wai ke za zhi* 1993;9(2):110-1, 59.
32. Kaufman T, Neuman RA, Weinberg A. Is postburn dermal ischaemia enhanced by oxygen free radicals? *Burns* 1989;15(5):291-4.
33. Lalonde C, Knox J, Youn YK, Demling R. Relationship between hepatic blood flow and tissue lipid peroxidation in the early postburn period. *Critical care medicine* 1992;20(6):789-96.
34. Buchanan M. You Can Prevent And Reverse Cancer. 2nd ed. USA: Xlibris Corporation; 2011.
35. Grune T. Oxidants and Antioxidant Defense Systems. Vol 2. USA: Springer; 2005.
36. Berger MM. Antioxidant micronutrients in major trauma and burns: evidence and practice. *Nutr Clin Pract* 2006;21(5):438-49.
37. Pintaudi AM, Tesoriere L, D'Arpa N, D'Amelio L, D'Arpa D, Bongiorno A, et al. Oxidative stress after moderate to extensive burning in humans. *Free radical research* 2000;33(2):139-46.
38. Halliwell B, Gutteridge J. Free Radicals in Biology and Medicine. 4th ed. London: Oxford university press; 2007.
39. Parihar A, Parihar MS, Milner S, Bhat S. Oxidative stress and anti-oxidative mobilization in burn injury. *Burns* 2008;34(1):6-17.
40. Koizumi T, Goto H, Tanaka H, Yamaguchi Y, Shimazaki S. Lecithinized superoxide dismutase suppresses free radical substrates during the early phase of burn care in rats. *J Burn Care Res* 2009;30(2):321-8.
41. Horton JW. Free radicals and lipid peroxidation mediated injury in burn trauma: the role of antioxidant therapy. *Toxicology* 2003;189(1-2):75-88.
42. Traber MG, Packer L. Vitamin E: beyond antioxidant function. *The American journal of clinical nutrition* 1995;62(6 Suppl):1501S-9S.
43. Makpol S, Azura Jam F, Anum Mohd Yusof Y, Zurinah Wan Ngah W. Modulation of collagen synthesis and its gene expression in human skin fibroblasts by tocotrienol-rich fraction. *Archives of medical science* 2011;7(5):889-95.
44. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free radical biology & medicine* 2007;43(1):4-15.
45. Niki E, Traber MG. A history of vitamin E. *Annals of nutrition & metabolism* 2012;61(3):207-12.
46. Traber MG. Does vitamin E decrease heart attack risk? summary and implications with respect to dietary recommendations. *J Nutr* 2001;131(2):395S-7S.

47. Vasanthi HR, Parameswari RP, Das DK. Tocotrienols and its role in cardiovascular health—a lead for drug design. *Current pharmaceutical design* 2011;17(21):2170-5.
48. Manca-di-Villahermosa S, Giacominielli-Stuffler R, Angelucci CB, Taccone-Gallucci M, Maccarrone M. Vitamin E-related inhibition of monocyte 5-lipoxygenase and cardiovascular outcome in maintenance hemodialysis patients. *Recent patents on inflammation & allergy drug discovery* 2011;5(3):229-40.
49. Shirpoor A, Ilkhanizadeh B, Saadatian R, Darvari BS, Behtaj F, Karimipour M, et al. Effect of vitamin E on diabetes-induced changes in small intestine and plasma antioxidant capacity in rat. *Journal of physiology and biochemistry* 2006;62(3):171-7.
50. Tsalie E, Kouzi K, Poutahidis T, Abas Z, Sarris K, Iliadis N, et al. Effect of vitamin E nutritional supplementation on the pathological changes induced in the ileum of rabbits by experimental infection with enteropathogenic *Escherichia coli*. *Journal of comparative pathology* 2006;134(4):308-19.
51. Nguyen TT, Cox CS, Traber DL, Gasser H, Redl H, Schlag G, et al. Free radical activity and loss of plasma antioxidants, vitamin E, and sulfhydryl groups in patients with burns: the 1993 Moyer Award. *The Journal of burn care & rehabilitation* 1993;14(6):602-9.
52. Wu X, Woodside KJ, Song J, Wolf SE. Burn-induced gut mucosal homeostasis in TCR delta receptor-deficient mice. *Shock (Augusta, Ga)* 2004;21(1):52-7.
53. Tu L, Fang HL, Su YP, Ai GP, Li X, Li M, et al. Influence of cervical sympathetic nerve block on blood flow volume and barrier function of intestinal mucosa after combined radiation and burn injury in rat. *Zhonghua shao shang za zhi Chinese journal of burns* 2007;23(3):208-11.
54. Jeschke MG, Herndon DN, Finnerty CC, Bolder U, Thompson JC, Mueller U, et al. The effect of growth hormone on gut mucosal homeostasis and cellular mediators after severe trauma. *The Journal of surgical research* 2005;127(2):183-9.
55. Shimoda N, Tashiro T, Yamamori H, Takagi K, Nakajima N, Ito I. Effects of growth hormone and insulin-like growth factor-1 on protein metabolism, gut morphology, and cell-mediated immunity in burned rats. *Nutrition* 1997;13(6):540-6.
56. Nafeeza MI, Kang TT. Synergistic effects of tocopherol, tocotrienol, and ubiquinone in indomethacin-induced experimental gastric lesions. *International journal for vitamin and nutrition research* 2005;75(2):149-55.
57. Yang YY, Lee TY, Huang YT, Chan CC, Yeh YC, Lee FY, et al. Asymmetric dimethylarginine (ADMA) determines the improvement of hepatic endothelial dysfunction by vitamin E in cirrhotic rats. *Liver international: official journal of the International Association for the Study of the Liver* 2012;32(1):48-57.
58. Khanna S, Parinandi NL, Kotha SR, Roy S, Rink C, Bibus D, et al. Nanomolar vitamin E alpha-tocotrienol inhibits glutamate-induced activation of phospholipase A2 and causes neuroprotection. *Journal of neurochemistry* 2010;112(5):1249-60.
59. Shoeb M, Ramana KV. Anti-inflammatory effects of benfotiamine are mediated through the regulation of the arachidonic acid pathway in macrophages. *Free radical biology & medicine* 2012;52(1):182-90.
60. Al-Malki AL, Moselhy S. Protective effect of vitamin E and epicatechin against nicotine-induced oxidative stress in rats. *Toxicology and industrial health* 2012.
61. Iranloye BO, Oludare GO. Garlic and vitamin E provides antioxidant defence in tissues of female rats treated with nicotine. *Nigerian journal of physiological sciences* 2011;26(1):103-7.
62. Berger MM, Soguel L, Shenkin A, Revelly JP, Pinget C, Baines M, et al. Influence of early antioxidant supplements on clinical evolution and organ function in critically ill cardiac surgery, major trauma, and subarachnoid hemorrhage patients. *Critical care (London, England)* 2008;12(4):R101.